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(54) Title: REGULATING METABOLISM BY MODIFYING THE LEVEL OF TREHALOSE-6-PHOSPHATE			
(57) Abstract The invention lies in the field of regulation of carbon flow in the metabolism of the cell. It has been found that induction of a change in the intracellular availability of the saccharide trehalose-6-phosphate (T-6-P) induces modifications of the development and/or composition of cells, tissue and organs <i>in vivo</i> . These changes can be induced by introducing or inhibiting the enzymes trehalose phosphate synthase (TPS) which is capable of forming T-6-P and trehalose phosphate phosphatase (TPP) which degrades T-6-P into trehalose. The carbon flow through the glycolysis will be stimulated by a decrease in the intracellular level of T-6-P.			

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**REGULATING METABOLISM BY MODIFYING THE LEVEL OF
TREHALOSE-6-PHOSPHATE**

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FIELD OF THE INVENTION

Glycolysis has been one of the first metabolic processes described in biochemical detail in the literature. Although the general flow of carbohydrates in organisms is known and although all enzymes of the glycolytic pathway(s) are elucidated, the signal which determines the induction of metabolism by stimulating glycolysis has not been unravelled. Several hypotheses, especially based on the situation in yeast have been put forward, but none has been proven beyond doubt.

Influence on the direction of the carbohydrate partitioning does not only influence directly the cellular processes of glycolysis and carbohydrate storage, but it can also be used to influence secondary or derived processes such as cell division, biomass generation and accumulation of storage compounds, thereby determining growth and productivity.

Especially in plants, often the properties of a tissue are directly influenced by the presence of carbohydrates, and the steering of carbohydrate partitioning can give substantial differences.

The growth, development and yield of plants depends on the energy which such plants can derive from CO₂-fixation during photosynthesis.

Photosynthesis primarily takes place in leaves and to a lesser extent in the stem, while other plant organs such as roots, seeds or tubers do not essentially contribute to the photoassimilation process. These tissues are completely dependent on photosynthetically active organs for their growth and nutrition. This then means that there is a flux of products derived from photosynthesis (collectively called "photosynthate") to photosynthetically inactive parts of the plants.

The photosynthetically active parts are denominated as "sources" and they are defined as net exporters of photosynthate. The photosynthetically inactive parts are denominated as "sinks" and they are defined as net importers of photosynthate.

It is assumed that both the efficiency of photosynthesis, as well as the carbohydrate partitioning in a plant are essential. Newly

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developing tissues like young leaves or other parts like root and seed are completely dependent on photosynthesis in the sources. The possibility of influencing the carbohydrate partitioning would have great impact on the phenotype of a plant, e.g. its height, the internodium distance, the size and form of a leaf and the size and structure of the root system.

Furthermore, the distribution of the photoassimilation products is of great importance for the yield of plant biomass and products. An example is the development in wheat over the last century. Its photosynthetic capacity has not changed considerably but the yield of wheat grain has increased substantially, i.e. the harvest index (ratio harvestable biomass/total biomass) has increased. The underlying reason is that the sink-to-source ratio was changed by conventional breeding, such that the harvestable sinks, i.e. seeds, portion increased. However, the mechanism which regulates the distribution of assimilation products and consequently the formation of sinks and sources is yet unknown. The mechanism is believed to be located somewhere in the carbohydrate metabolic pathways and their regulation. In the recent research it has become apparent that hexokinases may play a major role in metabolite signalling and control of metabolic flow. A number of mechanisms for the regulation of the hexokinase activity have been postulated (Graham et al. (1994), The Plant Cell 6: 761; Jang & Sheen (1994), The Plant Cell 6, 1665; Rose et al. Eur. J. Biochem. 199, 511-518, 1991; Blazquez et al. (1993), FEBS 329, 51; Koch, Annu. Rev. Plant Physiol. Plant. Mol. Biol. (1996) 47, 509; Jang et al. (1997), The Plant Cell 9, 5. One of these theories of hexokinase regulation, postulated in yeast mentions trehalose and its related monosaccharides (Thevelein & Hohmann (1995), TIBS 20, 3). However, it is hard to see that this would be an universal mechanism, as trehalose synthesis is believed to be restricted to certain species.

Thus, there still remains a need for the elucidation of the signal which can direct the modification of the development and/or composition of cells, tissue and organs *in vivo*.

SUMMARY OF THE INVENTION

It has now been found that modification of the development and/or composition of cells, tissue and organs *in vivo* is possible by
5 introducing the enzyme trehalose-6-phosphate synthase (TPS) and/or trehalose-6-phosphatase (TPP) thereby inducing a change in metabolic pathways of the saccharide trehalose-6-phosphate (T-6-P) resulting in an alteration of the intracellular availability of T-6-P. Introduction of TPS thereby inducing an increase in the intracellular
10 concentration of T-6-P causes inhibition of carbon flow in the glycolytic direction, stimulation of the photosynthesis, inhibition of growth, stimulation of sink-related activity and an increase in storage of resources. Introduction of TPP thereby introducing a decrease in the intracellular concentration of T-6-P causes
15 stimulation of carbon flow in the glycolytic direction, increase in biomass and a decrease in photosynthetic activity.

The levels of T-6-P may be influenced by genetic engineering of an organism with gene constructs able to influence the level of T-6-P or by exogenously (orally, topically, parenterally etc.) supplying
20 compounds able to influence these levels.

The gene constructs that can be used in this invention are constructs harbouring the gene for trehalose phosphate synthase (TPS) the enzyme that is able to catalyze the reaction from glucose-6-phosphate and UDP-glucose to T-6-P. On the other side a construct coding for the
25 enzyme trehalose-phosphate phosphatase (TPP) which catalyzes the reaction from T-6-P to trehalose will, upon expression, give a decrease of the amount of T-6-P.

Alternatively, gene constructs harbouring antisense TPS or TPP can be used to regulate the intracellular availability of T-6-P.

30 Furthermore, it was recently reported that an intracellular phospho-alpha-(1,1)-glucosidase, TreA, from *Bacillus subtilis* was able to hydrolyse T-6-P into glucose and glucose-6-phosphate (Schöck et al., Gene, 170, 77-80, 1996). A similar enzyme has already been described for *E. coli* (Rimmele and Boos (1996), J. Bact. 176 (18),
35 5654-).

For overexpression heterologous or homologous gene constructs have to be used. It is believed that the endogenous T-6-P forming and/or degrading enzymes are under allosteric regulation and

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regulation through covalent modification. This regulation may be circumvented by using heterologous genes.

Alternatively, mutation of heterologous or homologous genes may be used to abolish regulation.

5 The invention also gives the ability to modify source-sink relations and resource allocation in plants. The whole carbon economy of the plant, including assimilate production in source tissues and utilization in source tissues can be modified, which may lead to increased biomass yield of harvested products. Using this approach, increased yield potential can be realized, as well as improved harvest index and product quality. These changes in source tissues can lead to changes in sink tissues by for instance increased export of photosynthase. Conversely changes in sink tissue can lead to change in source tissue.

15 Specific expression in a cell organelle, a tissue or other part of an organism enables the general effects that have been mentioned above to be directed to specific local applications. This specific expression can be established by placing the genes coding for TPS, TPP or the antisense genes for TPS or TPP under control of a specific promoter.

20 Specific expression also enables the simultaneous expression of both TPS and TPP enzymes in different tissues thereby increasing the level of T-6-P and decreasing the level of T-6-P locally.

25 By using specific promoters it is also possible to construct a temporal difference. For this purpose promoters can be used that are specifically active during a certain period of the organogenesis of the plant parts. In this way it is possible to first influence the amount of organs which will be developed and then enable these organs to be filled with storage material like starch, oil or proteins.

30 Alternatively, inducible promoters may be used to selectively switch on or off the expression of the genes of the invention. Induction can be achieved by for instance pathogens, stress, chemicals or light/dark stimuli.

DEFINITIONS

- Hexokinase activity is the enzymatic activity found in cells which catalyzes the reaction of hexose to hexose-6-phosphate.
- 5 Hexoses include glucose, fructose, galactose or any other C₆ sugar. It is acknowledged that there are many isoenzymes which all can play a part in said biochemical reaction. By catalyzing this reaction hexokinase forms a key enzyme in hexose (glucose) signalling.
- 10 - Hexose signalling is the regulatory mechanism by which a cell senses the availability of hexose (glucose).
- Glycolysis is the sequence of reactions that converts glucose into pyruvate with the concomitant production of ATP.
- Cold sweetening is the accumulation of soluble sugars in potato
- 15 tubers after harvest when stored at low temperatures.
- Storage of resource material is the process in which the primary product glucose is metabolized into the molecular form which is fit for storage in the cell or in a specialized tissue. These forms can be divers. In the plant kingdom storage mostly takes
- 20 place in the form of carbohydrates and polycarbohydrates such as starch, fructan and cellulose, or as the more simple mono- and di-saccharides like fructose, sucrose and maltose; in the form of oils such as arachic or oleic oil and in the form of proteins such as cruciferin, napin and seed storage proteins in rapeseed.
- 25 In animal cells also polymeric carbohydrates such as glycogen are formed, but also a large amount of energy rich carbon compounds is transferred into fat and lipids.
- Biomass is the total mass of biological material.

DESCRIPTION OF THE FIGURES

- Figure 1. Schematic representation of plasmid pVDH275 harbouring the neomycin-phosphotransferase gene (NPTII) flanked by the 35S cauliflower mosaic virus promoter (P35S) and terminator (T35S) as a selectable marker; an expression cassette comprising the pea plastocyanin promoter (pPC_{pea}) and the nopaline synthase terminator (Tnos); right (RB) and left (LB) T-DNA border sequences and a bacterial kanamycin resistance (KanR) marker gene.
- Figure 2. Northern blot analysis of transgenic tobacco plants. Panel A depicts expression of *otsA* mRNA in leaves of individual pMOG799 transgenic tobacco plants. The control lane "C" contains total RNA from a non-transformed *N. tabacum* plant.
- Figure 3. Lineup of plant derived TPS encoding sequences compared with the TPS_{yeast} sequence using the Wisconsin GCG sequence analysis package (Devereux et al. (1984) A comprehensive set of sequence analysis programs of the VAX. Nucl. Acids Res., 12, 387).
- TPS_{satal} 3/56 and 142 TPS_{rice3} (SEQ ID NO:53) and RiceTPS code for respectively Arabidopsis and Rice TPS enzymes derived from EST database sequences.
- TPS_{sun10}, TPS_{sel43}, (SEQ ID NO:44) and TPS_{sel8} (SEQ ID NO:42) code for respectively sunflower and *Selaginella* TPS enzymes derived from sequences isolated by PCR techniques (see example 3).
- Figure 4. Alignment of PCR amplified tobacco TPS cDNA fragments with the TPS encoding yeast TPS1 gene. Boxes indicate identity between amino-acids of all four listed sequences.
- Figure 5. Alignment of PCR amplified tobacco TPP cDNA fragments with the TPP encoding yeast TPS2 gene. Boxes indicate identity between amino-acids of all four listed sequences.
- Figure 6. Alignment of a fragment of the PCR amplified sunflower TPS/TPP bipartite cDNA (SEQ ID NO: 24) with the TPP encoding yeast TPS2 gene. Boxes indicate identity between amino-acids of both sequences.

Figure 7. Alignment of a fragment of the *Arabidopsis* TPS1 and Rice EST clones with the TPS encoding yeast TPS1 gene. Boxes indicate identity between amino-acids of all three sequences.

- 5 Figure 8. Alignment of a fragment of the PCR amplified human TPS cDNA (SEQ ID NO: 10) with the TPS encoding yeast TPS1 gene. Boxes indicate identity between amino-acids of both sequences.

- Figure 9. Trehalose accumulation in tubers of pMOG1027 (35S as-
10 trehalase) transgenic potato plants.

- Figure 10. Hexokinase activity of a wild-type potato tuber (*Solanum tuberosum* cv. Kardal) extract with and without the addition of trehalose-6-phosphate.

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- Figure 11. Hexokinase activity of a wild-type potato tuber (*Solanum tuberosum* cv. Kardal) extract with and without the addition of trehalose-6-phosphate. Fructose or glucose is used as substrate for the assay.

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- Figure 12. Hexokinase activity of a wild-type tobacco leaf extract (*Nicotiana tabacum* cv. SR1) with and without the addition of trehalose-6-phosphate. Fructose or glucose is used as substrate for the assay.

25

- Figure 13. Plot of a tobacco hexokinase activity measurement.
Data series 1: Tobacco plant extract
Data series 2: Tobacco plant extract + 1 mM trehalose-6-phosphate
Data series 3: Commercial hexokinase extract from yeast (1/8 unit)

30

- Figure 14. Hexokinase activity of a wild-type rice leaf extract (*Oryza sativa*) extract with and without the addition of trehalose-6-phosphate. Experiments have been performed in duplicate using different amounts of extracts. Fructose or glucose is used as
35 substrate for the assay.

Figure 15. Hexokinase activity of a wild-type maize leaf extract (*Zea mais*) extract with and without the addition of trehalose-6-phosphate. Fructose or glucose is used as substrate for the assay.

- 5 Figure 16. Fluorescence characteristics of wild-type (triangle), PC-TPS (square) and 35S-TPP (cross) tobacco leaves. The upper two panels show the electron transport efficiency (ETE) at the indicated light intensities (PAR). Plants were measured after a dark-period (upper-left panel) and after a light-period (upper-right panel).
- 10 The bottom panels show reduction of fluorescence due to assimilate accumulation (non-photochemical quenching). Left and right panel as above.

- Figure 17. Relative sink-activity of plant-parts of PC-TPS (Famine) and 35S-TPP (Feast) transgenic tobacco plants. Indicated is the nett C-accumulation expressed as percentage of total C-content, for various plant-parts after a period of light (D) or light + dark (D + N).
- 15

- Figure 18. Actual distribution of carbon in plant-parts of PC-TPS (Famine) and 35S-TPP (Feast) transgenic tobacco plants. Indicated is the nett C-accumulation expressed as percentage of total daily accumulated new C for various plant-parts after a period of light (D) or light + dark (D + N).
- 20

- 25 Figure 19. Reduced and enhanced bolting in transgenic lettuce lines expressing PC-TPS or PC-TPP compared to wild-type plants. The lower panel shows leaf morphology and colour.

- Figure 20. Profile of soluble sugars (Fig. 20/1) in extracts of transgenic lettuce (upper panel) and transgenic beet (lower panel) lines. In the upper panel controls are GUS-transgenic lines which are compared to lines transgenics for PC-TPS and PC-TPP. In the lower panel all transgenic are PC-TPS. Starch profiles are depicted in Fig. 20/2.
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Figure 21. Plant and leaf morphology of transgenic sugarbeet lines expressing PC-TPS (TPS) or PC-TPP (TPP) compared to wild-type plants (Control). TPS A-type has leaves which are comparable to wild-type while TPS D-type has clearly smaller leaves. The leaves of the TPP transgenic line have a lighter green colour, a larger petiole and an increased size compared to the control.

Figure 22. Taproot diameter of transgenic sugarbeet lines (PC-TPS). In the upper panel A, B, C and D indicate decreasing leaf sizes as compared to control (A). In the lower panel individual clones of control and PC-TPS line 286-2 are shown.

Figure 23. Tuber yield of pMOG799 (35S TPS) transgenic potato lines.

Figure 24. Tuber yield of pMOG1010 (35S TPP) and pMOG1124 (PC-TPP) transgenic potato lines.

Figure 25. Tuber yield of 22 independent wild-type *S. tuberosum* clones.

Figure 26. Tuber yield of pMOG1093 (PC-TPS) transgenic potato lines in comparison to wild-type. B, C, D, E, F, G indicate decreasing leaf sizes as compared to wild-type (B/C).

Figure 27. Tuber yield of pMOG845 (Pat-TPS) transgenic potato lines (Figure 27-1) in comparison to wild-type (Figure 27-2). B, C indicate leaf sizes.

Figure 28. Tuber yield of pMOG1129 (845-11/22/28) transgenic potato lines.

Figure 29. Cross section through leaves of TPP (lower panel) and TPS (upper panel) transgenic tobacco plants. Additional cell layers and increased cell size are visible in the TPS cross section.

Figure 30. HPLC-PED analysis of tubers transgenic for TPS_{E.coli} before and after storage at 4°C. Kardal C, F, B, G and H are non-transgenic control lines.

- 5 Figure 31. Leaf morphology, colour and size of tobacco lines transgenic for 35S TPS (upper leaf), wild-type (middle leaf) and transgenic for 35S TPP (bottom leaf).

- Figure 32. Metabolic profiling of 35S TPS (pMOG799), 35S TPP (pMOG1010), wild-type (WT), PC-TPS (pMOG1177) and PC-TPP (pMOG1124) transgenic tobacco lines. Shown are the levels of trehalose, soluble sugars (Figure 32-1), starch and chlorophyll (Figure 32-2).

- Figure 33. Tuber yield of pMOG1027 (35S as-trehalase) and pMOG1027(845-11/22/28) (35S as-trehalase pat TPS) transgenic potato lines in comparison to wild-type potato lines.

- Figure 34. Starch content of pMOG1027 (35S as-trehalase) and pMOG1027(845-11/22/28) (35S as-trehalase pat TPS) transgenic potato lines in comparison to wild-type potato lines. The sequence of all lines depicted is identical to Fig. 33.

- Figure 35. Yield of pMOG1028 (pat as-trehalase) and pMOG1028(845-11/22/28) (pat as-trehalase pat TPS) transgenic potato lines in comparison to wild-type potato lines.

Figure 36. Yield of pMOG1092 (PC as-trehalase) transgenic potato lines in comparison to wild-type potato lines as depicted in Fig. 35.

- 30 Figure 37. Yield of pMOG1130 (PC as-trehalase PC TPS) transgenic potato lines in comparison to wild-type potato lines as depicted in Fig. 35.

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DETAILED DESCRIPTION OF THE INVENTION

The invention is concerned with the finding that metabolism can be modified *in vivo* by the level of T-6-P. A decrease of the intracellular concentration of T-6-P stimulates glycolytic activity.

5 On the contrary, an increase of the T-6-P concentration will inhibit glycolytic activity and stimulate photosynthesis.

These modifications established by changes in T-6-P levels are most likely a result of the signalling function of hexokinase, which activity is shown to be regulated by T-6-P. An increase in the flux
10 through hexokinase (i.e. an increase in the amount of glucose) that is reacted in glucose-6-phosphate has been shown to inhibit photosynthetic activity in plants. Furthermore, an increase in the flux through hexokinase would not only stimulate the glycolysis, but also cell division activity.

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THEORY OF TREHALOSE-6-PHOSPHATE REGULATION OF CARBON METABOLISM

In a normal plant cell formation of carbohydrates takes place in the process of photosynthesis in which CO₂ is fixed and reduced to
20 phosphorylated hexoses with sucrose as an end-product. Normally this sucrose is transported out of the cell to cells or tissues which through uptake of this sucrose can use the carbohydrates as building material for their metabolism or are able to store the carbohydrates as e.g. starch. In this respect, in plants, cells that are able to
25 photosynthesize and thus to produce carbohydrates are denominated as sources, while cells which consume or store the carbohydrates are called sinks.

In animal and most microbial cells no photosynthesis takes place and the carbohydrates have to be obtained from external sources,
30 either by direct uptake from saccharides (e.g. yeasts and other micro-organisms) or by digestion of carbohydrates (animals). Carbohydrate transport usually takes place in these organisms in the form of glucose, which is actively transported over the cell membrane.

After entrance into the cell, one of the first steps in the
35 metabolic pathway is the phosphorylation of glucose into glucose-6-phosphate catalyzed by the enzyme hexokinase. It has been demonstrated that in plants sugars which are phosphorylated by hexokinase (HXK) are controlling the expression of genes involved in photosynthesis (Jang &

Sheen (1994), The Plant Cell 6, 1665). Therefore, it has been proposed that HXK may have a dual function and may act as a key sensor and signal transmitter of carbohydrate-mediated regulation of gene-expression. It is believed that this regulation normally signals the cell about the availability of starting product, i.e. glucose. Similar effects are observed by the introduction of TPS or TPP which influence the level of T-6-P. Moreover, it is shown that *in vitro* T-6-P levels affect hexokinase activity. By increasing the level of T-6-P, the cell perceives a signal that there is a shortage of carbohydrate input. Conversely, a decrease in the level of T-6-P results in a signal that there is plenty of glucose, resulting in the down-regulation of photosynthesis: it signals that substrate for glycolysis and consequently energy supply for processes as cell growth and cell division is sufficiently available. This signalling is thought to be initiated by the increased flux through hexokinase (J.J. Van Oosten, public lecture at RijksUniversiteit Utrecht dated April 19, 1996).

The theory that hexokinase signalling in plants can be regulated through modulation of the level of trehalose-6-phosphate would imply that all plants require the presence of an enzyme system able to generate and break-down the signal molecule trehalose-6-phosphate. Although trehalose is commonly found in a wide variety of fungi, bacterial, yeasts and algae, as well as in some invertebrates, only a very limited range of vascular plants have been proposed to be able to synthesize this sugar (Elbein (1974), Adv. Carboh. Chem. Biochem. 30, 227). A phenomenon which was not understood until now is that despite the apparent lack of trehalose synthesizing enzymes, all plants do seem to contain trehalases, enzymes which are able to break down trehalose into two glucose molecules.

Indirect evidence for the presence of a metabolic pathway for trehalose is obtained by experiments presented herein with trehalase inhibitors such as Validamycin A or transformation with anti-sense trehalase.

Production of trehalose would be hampered if its intermediate T-6-P would influence metabolic activity too much. Preferably, in order to accumulate high levels of trehalose without affecting partitioning and allocation of metabolites by the action of trehalose-6-phosphate, one should overexpress a bipartite TPS/TPP enzyme. Such an enzyme would resemble a genetic constitution as found in yeast, where the

TPS2 gene product harbours a TPS and TPP homologous region when compared with the *E. coli* otsA and otsB gene (Kaasen et al. (1994), Gene 145, 9). Using such an enzyme, trehalose-6-phosphate will not become freely available to other cell components. Another example of such a bipartite enzyme is given by Zentella & Iturriaga (Plant Physiol. (1996), 111 Abstract 88) who isolated a 3.2 kb cDNA from *Selaginella lepidophylla* encoding a putative trehalose-6-phosphate synthase/phosphatase. It is also envisaged that construction of a truncated TPS-TPP gene product, whereby only the TPS activity would be retained, would be as powerful for synthesis of T-6-P as the otsA gene of *E. coli*, also when used in homologous systems.

On a molecular level we have data that indicate that next to *Selaginella* also trehalose synthesizing genes are present in *Arabidopsis*, tobacco, rice and sunflower. Using degenerated primers, based on conserved sequences between TPS_{E.coli} and TPS_{yeast}, we have been able to identify genes encoding putative trehalose-6-phosphate generating enzymes in sunflower and tobacco. Sequence comparison revealed significant homology between these sequences, the TPS genes from yeast and *E. coli*, and EST (expressed sequences tags) sequences from *Arabidopsis* and rice (see also Table 6b which contains the EST numbers of homologous EST's found).

Recently an *Arabidopsis* gene has been elucidated (disclosed in GENBANK Acc. No. Y08568, depicted in SEQ ID NO: 39) that on basis of its homology can be considered as a bipartite enzyme. These data indicate that, in contrast to current beliefs, most plants do contain genes which encode trehalose-phosphate-synthases enabling them to synthesize T-6-P. As proven by the accumulation of trehalose in TPS expressing plants, plants also contain phosphatases, non-specific or specific, able to dephosphorylate the T-6-P into trehalose. The presence of trehalase in all plants may be to effectuate turnover of trehalose.

Furthermore, we also provide data that T-6-P is involved in regulating carbohydrate pathways in human tissue. We have elucidated a human TPS gene (depicted in SEQ ID NO: 10) which shows homology with the TPS genes of yeast, *E. coli* and plants. Furthermore, we show data that also the activity of hexokinase is influenced in mammalian (mouse) tissue.

Generation of the "plenty" signal by decreasing the intracellular concentration of trehalose-6-phosphate through expression of the enzyme TPP (or inhibition of the enzyme TPS) will signal all cell systems to increase glycolytic carbon flow and inhibit
5 photosynthesis. This is nicely shown in the experimental part, where for instance in Experiment 2 transgenic tobacco plants are described in which the enzyme TPP is expressed having increased leaf size, increased branching and a reduction of the amount of chlorophyll. However, since the "plenty" signal is generated in the absence of
10 sufficient supply of glucose, the pool of carbohydrates in the cell is rapidly depleted.

Thus, assuming that the artificial "plenty" signal holds on, the reduction in carbohydrates will finally become limiting for growth and cell division, i.e. the cells will use up all their storage
15 carbohydrates and will be in a "hunger"-stage. Thus, leaves are formed with a low amount of stored carbohydrates. On the other hand, plants that express a construct with a gene coding for TPS, which increases the intracellular amount of T-6-P, showed a reduction of leaf size, while also the leaves were darker green, and contained an increased
20 amount of chlorophyll.

In yeast, a major role of glucose-induced signalling is to switch metabolism from a neogenetic/respirative mode to a fermentative mode. Several signalling pathways are involved in this phenomenon (Thevelein
25 and Hohmann, (1995) TIBS 20, 3). Besides the possible role of hexokinase signalling, the RAS-cyclic-AMP (cAMP) pathway has been shown to be activated by glucose. Activation of the RAS-cAMP pathway by glucose requires glucose phosphorylation, but no further glucose metabolism. So far, this pathway has been shown to activate trehalase
30 and 6-phosphofructo-2-kinase (thereby stimulating glycolysis), while fructose-1,6-bisphosphatase is inhibited (thereby preventing gluconeogenesis), by cAMP-dependent protein phosphorylation. This signal transduction route and the metabolic effects it can bring about can thus be envisaged as one that acts in parallels with the
35 hexokinase signalling pathway, that is shown to be influenced by the level of trehalose-6-phosphate.

As described in our invention, transgenic plants expressing as-trehalase reveal similar phenomena, like dark-green leaves, enhanced

yield, as observed when expressing a TPS gene. It also seems that expression of as-trehalase in double-constructs enhances the effects that are caused by the expression of TPS. Trehalase activity has been shown to be present in e.g. plants, insects, animals, fungi and
5 bacteria while only in a limited number of species, trehalose is accumulated.

Up to now, the role of trehalase in plants is unknown although this enzyme is present in almost all plant-species. It has been proposed to be involved in plant pathogen interactions and/or plant defense
10 responses. We have isolated a potato trehalase gene and show that inhibition of trehalase activity in potato leaf and tuber tissues leads to an increase in tuber-yield. Fruit-specific expression of as-trehalase in tomato combined with TPS expression dramatically alters fruit development.

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According to one embodiment of the invention, accumulation of T-6-P is brought about in cells in which the capacity of producing T-6-P has been introduced by introduction of an expressible gene construct encoding trehalose-phosphate-synthase (TPS). Any trehalose phosphate
20 synthase gene under the control of regulatory elements necessary for expression of DNA in cells, either specifically or constitutively, may be used, as long as it is capable of producing a trehalose phosphate synthase capable of T-6-P production in said cells. One example of an open reading frame according to the invention is one encoding a TPS-
25 enzyme as represented in SEQ ID NO: 2. Other examples are the open reading frames as represented in SEQ ID NO's: 10, 18-23, 41 and 45-53. As is illustrated by the above-mentioned sequences it is well known that more than one DNA sequence may encode an identical enzyme, which fact is caused by the degeneracy of the genetic code. If desired, the
30 open reading frame encoding the trehalose phosphate synthase activity may be adapted to codon usage in the host of choice, but this is not a requirement.

The isolated nucleic acid sequence represented by for instance SEQ ID NO: 2, may be used to identify trehalose phosphate synthase
35 genes in other organisms and subsequently isolating and cloning them, by PCR techniques and/or by hybridizing DNA from other sources with a DNA- or RNA fragment obtainable from the *E. coli* gene. Preferably, such DNA sequences are screened by hybridizing under more or less

stringent conditions (influenced by factors such as temperature and ionic strength of the hybridization mixture). Whether or not conditions are stringent also depends on the nature of the hybridization, *i.e.* DNA:DNA, DNA:RNA, RNA:RNA, as well as the length of the shortest hybridizing fragment. Those of skill in the art are readily capable of establishing a hybridization regime stringent enough to isolate TPS genes, while avoiding non-specific hybridization. As genes involved in trehalose synthesis from other sources become available these can be used in a similar way to obtain an expressible trehalose phosphate synthase gene according to the invention. More detail is given in the experimental section.

Sources for isolating trehalose phosphate synthase activities include microorganisms (*e.g.* bacteria, yeast, fungi), plants, animals, and the like. Isolated DNA sequences encoding trehalose phosphate synthase activity from other sources may be used likewise in a method for producing T-6-P according to the invention. As an example, genes for producing T-6-P from yeast are disclosed in WO 93/17093.

The invention also encompasses nucleic acid sequences which have been obtained by modifying the nucleic acid sequence represented in SEQ ID NO: 1 by mutating one or more codons so that it results in amino acid changes in the encoded protein, as long as mutation of the amino acid sequence does not entirely abolish trehalose phosphate synthase activity.

According to another embodiment of the invention the trehalose-6-phosphate in a cell can be converted into trehalose by trehalose phosphate phosphatase encoding genes under control of regulatory elements necessary for the expression of DNA in cells. A preferred open reading frame according to the invention is one encoding a TPP-enzyme as represented in SEQ ID NO: 4 (Kaasen *et al.* (1994) *Gene*, 145, 9). It is well known that more than one DNA sequence may encode an identical enzyme, which fact is caused by the degeneracy of the genetic code. If desired, the open reading frame encoding the trehalose phosphate phosphatase activity may be adapted to codon usage in the host of choice, but this is not a requirement.

The isolated nucleic acid sequence represented by SEQ ID NO: 3, may be used to identify trehalose phosphate phosphatase genes in other organisms and subsequently isolating and cloning them, by PCR techniques and/or by hybridizing DNA from other sources with a DNA- or

RNA fragment obtainable from the *E. coli* gene. Preferably, such DNA sequences are screened by hybridizing under more or less stringent conditions (influenced by factors such as temperature and ionic strength of the hybridization mixture). Whether or not conditions are stringent also depends on the nature of the hybridization, i.e. DNA:DNA, DNA:RNA, RNA:RNA, as well as the length of the shortest hybridizing fragment. Those of skill in the art are readily capable of establishing a hybridization regime stringent enough to isolate TPP genes, while avoiding aspecific hybridization. As genes involved in trehalose synthesis from other sources become available these can be used in a similar way to obtain an expressible trehalose phosphate phosphatase gene according to the invention. More detail is given in the experimental section.

Sources for isolating trehalose phosphate phosphatase activities include microorganisms (e.g. bacteria, yeast, fungi), plants, animals, and the like. Isolated DNA sequences encoding trehalose phosphate phosphatase activity from other sources may be used likewise.

The invention also encompasses nucleic acid sequences which have been obtained by modifying the nucleic acid sequence represented in SEQ ID NO: 3 by mutating one or more codons so that it results in amino acid changes in the encoded protein, as long as mutation of the amino acid sequence does not entirely abolish trehalose phosphate phosphatase activity.

Other enzymes with TPS or TPP activity are represented by the so-called bipartite enzymes. It is envisaged that the part of the sequence which is specifically coding for one of the two activities can be separated from the part of the bipartite enzyme coding for the other activity. One way to separate the activities is to insert a mutation in the sequence coding for the activity that is not selected, by which mutation the expressed protein is impaired or deficient of this activity and thus only performs the other function. This can be done both for the TPS- and TPP-activity coding sequence. Thus, the coding sequences obtained in such a way can be used for the formation of novel chimaeric open reading frames capable of expression of enzymes having either TPS or TPP activity.

According to another embodiment of the invention, especially plants can be genetically altered to produce and accumulate the above-mentioned enzymes in specific parts of the plant. Preferred sites of

enzyme expression are leaves and storage parts of plants. In particular potato tubers are considered to be suitable plant parts. A preferred promoter to achieve selective TPS-enzyme expression in microtubers and tubers of potato is obtainable from the region
5 upstream of the open reading frame of the patatin gene of potato.

Another suitable promoter for specific expression is the plastocyanin promoter, which is specific for photoassimilating parts of plants. Furthermore, it is envisaged that specific expression in plant parts can yield a favourable effect for plant growth and
10 reproduction or for economic use of said plants. Promoters which are useful in this respect are: the E8-promoter (EP 0 409 629) and the 2A11-promoter (van Haaren and Houck (1993), Plant Mol. Biol., 221, 625) which are fruit-specific; the cruciferin promoter, the napin promoter and the ACP promoter which are seed-specific; the PAL-
15 promoter; the chalcon-isomerase promoter which is flower-specific; the SSU promoter, and ferredoxin promoter, which are leaf-specific; the TobRb7 promoter which is root-specific, the RolC promoter which is specific for phloem and the HMG2 promoter (Enjuto et al. (1995), Plant Cell 7, 517) and the rice PCNA promoter (Kosugi et al. (1995), Plant
20 J. 7, 877) which are specific for meristematic tissue.

Another option under this invention is to use inducible promoters. Promoters are known which are inducible by pathogens, by stress, by chemical or light/dark stimuli. It is envisaged that for induction of specific phenomena, for instance sprouting, bolting, seed
25 setting, filling of storage tissues, it is beneficial to induce the activity of the genes of the invention by external stimuli. This enables normal development of the plant and the advantages of the inducibility of the desired phenomena at control. Promoters which qualify for use in such a regime are the pathogen inducible promoters
30 described in DE 4446342 (fungus and auxin inducible PRP-1), WO 96/28561 (fungus inducible PRP-1), EP 0 586 612 (nematode inducible), EP 0 712 273 (nematode inducible), WO 96/34949 (fungus inducible), PCT/EP96/02437 (nematode inducible), EP 0 330 479 (stress inducible), US 5,510,474 (stress inducible), WO 96/12814 (cold inducible), EP 0
35 494 724 (tetracycline inducible), EP 0 619 844 (ethylene inducible), EP 0 337 532 (salicylic acid inducible), WO 95/24491 (thiamine inducible) and WO 92/19724 (light inducible). Other chemical inducible promoters are described in EP 0 674 608, EP 637 339, EP 455 667 and US

5,364,780.

According to another embodiment of the invention, cells are transformed with constructs which inhibit the function of the endogenously expressed TPS or TPP. Inhibition of undesired endogenous enzyme activity is achieved in a number of ways, the choice of which is not critical to the invention. One method of inhibition of gene expression is achieved through the so-called 'antisense approach'. Herein a DNA sequence is expressed which produces an RNA that is at least partially complementary to the RNA which encodes the enzymatic activity that is to be blocked. It is preferred to use homologous antisense genes as these are more efficient than heterologous genes. An alternative method to block the synthesis of undesired enzymatic activities is the introduction into the genome of the plant host of an additional copy of an endogenous gene present in the plant host. It is often observed that such an additional copy of a gene silences the endogenous gene: this effect is referred to in the literature as the co-suppressive effect, or co-suppression. Details of the procedure of enhancing substrate availability are provided in the Examples of WO 95/01446, incorporated by reference herein.

Host cells can be any cells in which the modification of hexokinase-signalling can be achieved through alterations in the level of T-6-P. Thus, accordingly, all eukaryotic cells are subject to this invention. From an economic point of view the cells most suited for production of metabolic compounds are most suitable for the invention. These organisms are, amongst others, plants, animals, yeast, fungi. However, also expression in specialized animal cells (like pancreatic beta-cells and fat cells) is envisaged.

Preferred plant hosts among the *Spermatophytæ* are the *Angiospermae*, notably the *Dicotyledoneae*, comprising *inter alia* the *Solanaceae* as a representative family, and the *Monocotyledoneae*, comprising *inter alia* the *Gramineae* as a representative family. Suitable host plants, as defined in the context of the present invention include plants (as well as parts and cells of said plants) and their progeny which contain a modified level of T-6-P, for instance by using recombinant DNA techniques to cause or enhance production of TPS or TPP in the desired plant or plant organ. Crops according to the invention include those which have flowers such as cauliflower (*Brassica oleracea*), artichoke (*Cynara scolymus*), cut

flowers like carnation (*Dianthus caryophyllus*), rose (*Rosa spp.*),
Chrysanthemum, *Petunia*, *Alstromeria*, *Gerbera*, *Gladiolus*, lily (*Lilium*
spp.), hop (*Humulus lupulus*), broccoli, potted plants like
Rhododendron, *Azalia*, *Dahlia*, *Begonia*, *Fuchsia*, *Geranium* etc.; fruits
5 such as apple (*Malus, e.g. domestica*), banana (*Musa, e.g. Acuminata*),
apricot (*Prunus armeniaca*), olive (*Oliva sativa*), pineapple (*Ananas*
comosus), coconut (*Cocos nucifera*), mango (*Mangifera indica*), kiwi,
avocado (*Persea americana*), berries (such as the currant, *Ribes, e.g.*
rubrum), cherries (such as the sweet cherry, *Prunus, e.g. avium*),
10 cucumber (*Cucumis, e.g. sativus*), grape (*Vitis, e.g. vinifera*), lemon
(*Citrus limon*), melon (*Cucumis melo*), mustard (*Sinapis alba* and
Brassica nigra), nuts (such as the walnut, *Juglans, e.g. regia*;
peanut, *Arachis hypogaeae*), orange (*Citrus, e.g. maxima*), peach
(*Prunus, e.g. persica*), pear (*Pyra, e.g. Communis*), pepper (*Solanum,*
15 *e.g. capsicum*), plum (*Prunus, e.g. domestica*), strawberry (*Fragaria,*
e.g. moschata), tomato (*Lycopersicon, e.g. esculentum*); leaves, such
as alfalfa (*Medicago sativa*), cabbages (such as *Brassica oleracea*),
endive (*Cichoreum, e.g. endivia*), leek (*Allium porrum*), lettuce
(*Lactuca sativa*), spinach (*Spinacia oleraceae*), tobacco (*Nicotiana*
20 *tabacum*), grasses like *Festuca*, *Poa*, rye-grass (such as *Lolium*
perenne, *Lolium multiflorum* and *Arrhenatherum spp.*), amenity grass,
turf, seaweed, chicory (*Cichorium intybus*), tea (*Thea sinensis*),
celery, parsley (*Petroselinum crispum*), chevil and other herbs; roots,
such as arrowroot (*Maranta arundinacea*), beet (*Beta vulgaris*), carrot
25 (*Daucus carota*), cassava (*Manihot esculenta*), ginseng (*Panax ginseng*),
turnip (*Brassica rapa*), radish (*Raphanus sativus*), yam (*Dioscorea*
esculenta), sweet potato (*Ipomoea batatas*), taro; seeds, such as beans
(*Phaseolus vulgaris*), pea (*Pisum sativum*), soybean (*Glycin max*), wheat
(*Triticum aestivum*), barley (*Hordeum vulgare*), corn (*Zea mays*), rice
30 (*Oryza sativa*), bush beans and broad beans (*Vicia faba*), cotton
(*Gossypium spp.*), coffee (*Coffea arabica* and *C. canephora*); tubers,
such as kohlrabi (*Brassica oleraceae*), potato (*Solanum tuberosum*);
bulbous plants as onion (*Allium cepa*), scallion, tulip (*Tulipa spp.*),
daffodil (*Narcissus spp.*), garlic (*Allium sativum*); stems such as
35 cork-oak, sugarcane (*Saccharum spp.*), sisal (*Sisal spp.*), flax (*Linum*
vulgare), jute; trees like rubber tree, oak (*Quercus spp.*), beech
(*Betula spp.*), alder (*Alnus spp.*), ashtree (*Acer spp.*), elm (*Ulmus*
spp.), palms, ferns, ivies and the like.

Transformation of yeast and fungal or animal cells can be done through normal state-of-the art transformation techniques through commonly known vector systems like pBluescript, pUC and viral vector systems like RSV and SV40.

5 The method of introducing the expressible trehalose-phosphate synthase gene, the expressible trehalose-phosphate-phosphatase gene, or any other sense or antisense gene into a recipient plant cell is not crucial, as long as the gene is expressed in said plant cell.

10 Although some of the embodiments of the invention may not be practicable at present, e.g. because some plant species are as yet recalcitrant to genetic transformation, the practicing of the invention in such plant species is merely a matter of time and not a matter of principle, because the amenability to genetic transformation as such is of no relevance to the underlying embodiment of the
15 invention.

Transformation of plant species is now routine for an impressive number of plant species, including both the *Dicotyledoneae* as well as the *Monocotyledoneae*. In principle any transformation method may be used to introduce chimeric DNA according to the invention into a
20 suitable ancestor cell. Methods may suitably be selected from the calcium/polyethylene glycol method for protoplasts (Krens et al. (1982), Nature 296, 72; Negrutiu et al. (1987), Plant Mol. Biol. 8, 363, electroporation of protoplasts (Shillito et al. (1985) Bio/Technol. 3, 1099), microinjection into plant material (Crossway et
25 al. (1986), Mol. Gen. Genet. 202), (DNA or RNA-coated) particle bombardment of various plant material (Klein et al. (1987), Nature 327, 70), infection with (non-integrative) viruses, in planta *Agrobacterium tumefaciens* mediated gene transfer by infiltration of adult plants or transformation of mature pollen or microspores (EP 0
30 301 316) and the like. A preferred method according to the invention comprises *Agrobacterium*-mediated DNA transfer. Especially preferred is the use of the so-called binary vector technology as disclosed in EP A 120 516 and U.S. Patent 4,940,838).

35 Although considered somewhat more recalcitrant towards genetic transformation, monocotyledonous plants are amenable to transformation and fertile transgenic plants can be regenerated from transformed cells or embryos, or other plant material. Presently, preferred methods for transformation of monocots are microprojectile bombardment

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of embryos, explants or suspension cells, and direct DNA uptake or (tissue) electroporation (Shimamoto et al. (1989), Nature 338, 274-276). Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus* bar-gene, which encodes
5 phosphinothricin acetyltransferase (an enzyme which inactivates the herbicide phosphinothricin), into embryogenic cells of a maize suspension culture by microprojectile bombardment (Gordon-Kamm (1990), Plant Cell, 2, 603). The introduction of genetic material into
10 aleurone protoplasts of other monocot crops such as wheat and barley has been reported (Lee (1989), Plant Mol. Biol. 13, 21). Wheat plants have been regenerated from embryogenic suspension culture by selecting embryogenic callus for the establishment of the embryogenic suspension cultures (Vasil (1990) Bio/Technol. 8, 429). The combination with
15 transformation systems for these crops enables the application of the present invention to monocots.

Monocotyledonous plants, including commercially important crops such as rice and corn are also amenable to DNA transfer by *Agrobacterium* strains (vide WO 94/00977; EP 0 159 418 B1; Gould et al. (1991) Plant. Physiol. 95, 426-434).

20 To obtain transgenic plants capable of constitutively expressing more than one chimeric gene, a number of alternatives are available including the following:

A. The use of DNA, e.g. a T-DNA on a binary plasmid, with a number of modified genes physically coupled to a second selectable marker gene.

25 The advantage of this method is that the chimeric genes are physically coupled and therefore migrate as a single Mendelian locus.

B. Cross-pollination of transgenic plants each already capable of expressing one or more chimeric genes, preferably coupled to a selectable marker gene, with pollen from a transgenic plant which
30 contains one or more chimeric genes coupled to another selectable marker. Afterwards the seed, which is obtained by this crossing, maybe selected on the basis of the presence of the two selectable markers, or on the basis of the presence of the chimeric genes themselves. The plants obtained from the selected seeds can afterwards be used for
35 further crossing. In principle the chimeric genes are not on a single locus and the genes may therefore segregate as independent loci.

C. The use of a number of a plurality chimeric DNA molecules, e.g. plasmids, each having one or more chimeric genes and a selectable

marker. If the frequency of co-transformation is high, then selection on the basis of only one marker is sufficient. In other cases, the selection on the basis of more than one marker is preferred.

D. Consecutive transformation of transgenic plants already containing
5 a first, second, (etc), chimeric gene with new chimeric DNA, optionally comprising a selectable marker gene. As in method B, the chimeric genes are in principle not on a single locus and the chimeric genes may therefore segregate as independent loci.

E. Combinations of the above mentioned strategies.

10 The actual strategy may depend on several considerations as maybe easily determined such as the purpose of the parental lines (direct growing, use in a breeding programme, use to produce hybrids) but is not critical with respect to the described invention.

It is known that practically all plants can be regenerated from
15 cultured cells or tissues. The means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Shoots may be induced directly, or indirectly from callus via organogenesis or embryogenesis and
20 subsequently rooted. Next to the selectable marker, the culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the
25 genotype and on the history of the culture. If these three variables are controlled regeneration is usually reproducible and repeatable. After stable incorporation of the transformed gene sequences into the transgenic plants, the traits conferred by them can be transferred to other plants by sexual crossing. Any of a number of standard breeding
30 techniques can be used, depending upon the species to be crossed.

Suitable DNA sequences for control of expression of the plant expressible genes (including marker genes), such as transcriptional initiation regions, enhancers, non-transcribed leaders and the like, may be derived from any gene that is expressed in a plant cell. Also
35 intended are hybrid promoters combining functional portions of various promoters, or synthetic equivalents thereof. Apart from constitutive promoters, inducible promoters, or promoters otherwise regulated in their expression pattern, e.g. developmentally or cell-type specific,

may be used to control expression of the expressible genes according to the invention.

To select or screen for transformed cells, it is preferred to include a marker gene linked to the plant expressible gene according to the invention to be transferred to a plant cell. The choice of a suitable marker gene in plant transformation is well within the scope of the average skilled worker; some examples of routinely used marker genes are the neomycin phosphotransferase genes conferring resistance to kanamycin (EP-B 131 623), the glutathion-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides (EP-A 256 223), glutamine synthetase conferring upon overexpression resistance to glutamine synthetase inhibitors such as phosphinothricin (WO 87/05327), the acetyl transferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin (EP-A 275 957), the gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine, the *bar* gene conferring resistance against Bialaphos (e.g. WO 91/02071) and the like. The actual choice of the marker is not crucial as long as it is functional (i.e. selective) in combination with the plant cells of choice.

The marker gene and the gene of interest do not have to be linked, since co-transformation of unlinked genes (U.S. Patent 4,399,216) is also an efficient process in plant transformation.

Preferred plant material for transformation, especially for dicotyledonous crops are leaf-discs which can be readily transformed and have good regenerative capability (Horsch et al. (1985), Science 227, 1229).

Specific use of the invention is envisaged in the following ways: as can be seen from the Examples the effects of the expression of TPP (which causes a decrease in the intracellular T-6-P concentration) are an increased leaf size, increased branching leading to an increase in the number of leaves, increase in total leaf biomass, bleaching of mature leaves, formation of more small flowers and sterility. These effects are specifically useful in the following cases: increased leaf size (and increase in the number of leaves) is economically important for leafy vegetables such as spinach, lettuce, leek, alfalfa, silage maize; for ground coverage and weed control by grasses and garden plants; for crops in which the leaves are used as

product, such as tobacco, tea, hemp and roses (perfumes!); for the matting up of cabbage-like crops such as cauliflower.

An additional advantage of the fact that these leaves are stimulated in their metabolic activity is that they tend to burn all
5 their intracellular resources, which means that they are low in starch-content. For plants meant for consumption a reduction in starch content is advantageous in the light of the present tendency for low-calorie foodstuffs. Such a reduction in starch content also has effects on taste and texture of the leaves. An increase in the
10 protein/carbohydrate balance as can be produced by the expression of TPP is especially important for leafy crops as silage maize.

Increased branching, which is accompanied by a tendency to have stems with a larger diameter, can be advantageous in crops in which the stem is responsible for the generation of an economically
15 attractive product. Examples in this category are all trees for the increased production of wood, which is also a starting material for paper production; crops like hemp, sisal, flax which are used for the production of rope and linen; crops like bamboo and sugarcane; rubber-tree, cork-oak; for the prevention of flattening in crops or crop
20 parts, like grains, corn, legumes and strawberries.

A third phenomenon is increased bleaching of the leaves (caused by a decrease of photosynthetic activity). Less colourful leaves are preferred for crops such as chicory and asparagus. Also for cut flowers bleaching in the petals can be desired, for instance in
25 *Alstromeria*.

An overall effect is the increase in biomass resulting from an increase in metabolic activity. This means that the biomass consists of metabolized compounds such as proteins and fats. Accordingly, there is an increased protein/carbohydrate balance in mature leaves which is
30 an advantage for crops like silage maize, and all fodder which can be ensilaged. A similar increased protein/carbohydrate balance can be established in fruits, tubers and other edible plant parts.

Outside the plant kingdom an increased metabolism would be beneficial for protein production in microorganisms or eukaryotic cell
35 cultures. Both production of endogenous but also of heterologous proteins will be enhanced which means that the production of heterologous proteins in cultures of yeast or other unicellular organisms can be enhanced in this way. For yeast this would give a

more efficient fermentation, which would result in an increased alcohol yield, which of course is favourable in brewery processes, alcohol production and the like.

In animals or human beings it is envisaged that diseases caused by a defect in metabolism can be overcome by stable expression of TPP or TPS in the affected cells. In human cells, the increased glucose consumption of many tumour cells depends to a large extent on the overexpression of hexokinase (Rempel et al. (1996) FEBS Lett. 385, 233). It is envisaged that the flux of glucose into the metabolism of cancer cells can be influenced by the expression of trehalose-6-phosphate synthesizing enzymes. It has also been shown that the hexokinase activation is potentiated by the cAMP/PKA (protein kinase A pathway). Therefore, inactivation of this signal transduction pathway may affect glucose uptake and the proliferation of neoplasias. Enzyme activities in mammalian cells able to synthesize trehalose-6-phosphate and trehalose and degrade trehalose have been shown in e.g. rabbit kidney cortex cells (Sacktor (1968) Proc. Natl.Acad.Sci. USA 60, 1007).

Another example can be found in defects in insulin secretion in pancreatic beta-cells in which the production of glucose-6-phosphate catalyzed by hexokinase is the predominant reaction that couples rises in extracellular glucose levels to insulin secretion (Efrat et al. (1994), TIBS 19, 535). An increase in hexokinase activity caused by a decrease of intracellular T-6-P then will stimulate insulin production in cells which are deficient in insulin secretion.

Also in transgenic animals an increased protein/carbohydrate balance can be advantageous. Both the properties of an increased metabolism and an enhanced production of proteins are of large importance in farming in which animals should gain in flesh as soon as possible. Transformation of the enzyme TPP into meat-producing animals like chickens, cattle, sheep, turkeys, goats, fish, lobster, crab, shrimps, snails etc, will yield animals that grow faster and have a more proteinaceous meat.

In the same way this increased metabolism means an increase in the burn rate of carbohydrates and it thus prevents obesity.

More plant-specific effects from the decrease of intracellular T-6-P concentration are an increase in the number of flowers (although they do not seem to lead to the formation of seed). However, an increase in the number of flowers is advantageous for cutflower plants and pot flower plants and also for all plants suitable for horticulture.

A further effect of this flowering phenomenon is sterility, because the plants do not produce seed. Sterile plants are advantageous in hybrid breeding.

Another economically important aspect is the prohibiting of bolting of culture crops such as lettuce, endive and both recreational and fodder grasses. This is a beneficial property because it enables the crop to grow without having to spend metabolic efforts to flowering and seed production. Moreover, in crops like lettuce, endive and grasses the commercial product/application is non-bolted.

Specific expression of TPP in certain parts (sinks) of the plant can give additional beneficial effects. It is envisaged that expression of TPP by a promoter which is active early in e.g. seed forming enables an increased growth of the developing seed. A similar effect would be obtained by expressing TPP by a flower-specific promoter. To put it shortly: excessive growth of a certain plant part is possible if TPP is expressed by a suitable specific promoter. In fruits specific expression can lead to an increased growth of the skin in relation to the flesh. This enables improvement of the peeling of the fruit, which can be advantageous for automatic peeling industries.

Expression of TPP during the process of germination of oil-storing seeds prevents oil-degradations. In the process of germination, the glyoxylate cycle is very active. This metabolic pathway converts acetyl-CoA via malate into sucrose which can be transported and used as energy source during growth of the seedling. Key-enzymes in this process are malate synthase and isocitrate lyase. Expression of both enzymes is supposed to be regulated by hexokinase signalling. One of the indications for this regulation is that both 2-deoxyglucose and mannose are phosphorylated by hexokinase and able to transduce their signal, being reduction of malate synthase and isocitrate lyase expression, without being further metabolised. Expression of TPP in the seed, thereby decreasing the inhibition of

hexokinase, thereby inhibiting malate synthase and isocitrate lyase maintains the storage of oil into the seeds and prevents germination.

In contrast to the effects of TPP the increase in T-6-P caused
5 by the expression of TPS causes other effects as is illustrated in the Examples. From these it can be learnt that an increase in the amount of T-6-P causes dwarfing or stunted growth (especially at high expression of TPS), formation of more lancet-shaped leaves, darker colour due to an increase in chlorophyll and an increase in starch
10 content. As is already acknowledged above, the introduction of an anti-sense trehalase construct will also stimulate similar effects as the introduction of TPS. Therefore, the applications which are shown or indicated for TPS will equally be established by using as-trehalase. Moreover, the use of double-constructs of TPS and as-
15 trehalase enhances the effects of a single construct.

Dwarfing is a phenomenon that is desired in horticultural plants, of which the Japanese bonsai trees are a proverbial example. However, also creation of mini-flowers in plants like allseed, roses,
20 Amaryllis, Hortensia, birch and palm will have economic opportunities. Next to the plant kingdom dwarfing is also desired in animals. It is also possible to induce bolting in culture crops such as lettuce. This is beneficial because it enables a rapid production of seed. Ideally the expression of TPS for this effect should be under
25 control of an inducible promoter.

Loss of apical dominance also causes formation of multiple shoots which is of economic importance for instance in alfalfa.

A reduction in growth is furthermore desired for the industry of "veggie snacks", in which vegetables are considered to be consumed in
30 the form of snacks. Cherry-tomatoes is an example of reduced size vegetables which are successful in the market. It can be envisaged that also other vegetables like cabbages, cauliflower, carrot, beet and sweet potato and fruits like apple, pear, peach, melon, and several tropical fruits like mango and banana would be marketable on
35 miniature size.

Reduced growth is desired for all cells that are detrimental to an organism, such as cells of pathogens and cancerous cells. In this last respect a role can be seen in regulation of the growth by

2.9

changing the level of T-6-P. An increase in the T-6-P level would reduce growth and metabolism of cancer tissue. One way to increase the intracellular level of T-6-P is to knock-out the TPP gene of such cells by introducing a specific recombination event which causes the introduction of a mutation in the endogenous TPP-genes. One way in which this could be done is the introduction of a DNA-sequence able of introducing a mutation in the endogenous gene via a cancer cell specific internalizing antibody. Another way is targeted microparticle bombardment with said DNA. Thirdly a cancer cell specific viral vectors having said DNA can be used.

The phenomenon of a darker green colour seen with an increased concentration of T-6-P, is a property which is desirable for pot flower plants and, in general, for species in horticulture and for recreational grasses.

Increase in the level of T-6-P also causes an increase in the storage carbohydrates such as starch and sucrose. This then would mean that tissues in which carbohydrates are stored would be able to store more material. This can be illustrated by the Examples where it is shown that in plants increased biomass of storage organs such as tubers and thickened roots as in beets (storage of sucrose) are formed.

Crops in which this would be very advantageous are potato, sugarbeet, carrot, chicory and sugarcane.

An additional economically important effect in potatoes is that after transformation with DNA encoding for the TPS gene (generating an increase in T-6-P) it has been found that the amount of soluble sugars decreases, even after harvest and storage of the tubers under cold conditions (4°C). Normally even colder storage would be necessary to prevent early sprouting, but this results in excessive sweetening of the potatoes. Reduction of the amount of reducing sugars is of major importance for the food industry since sweetened potato tuber material is not suitable for processing because a Maillard reaction will take place between the reducing sugars and the amino-acids which results in browning.

In the same way also inhibition of activity of invertase can be obtained by transforming sugarbeets with a polynucleotide encoding for the enzyme TPS. Inhibition of invertase activity in sugarbeets after

harvest is economically very important.

Also in fruits and seeds, storage can be altered. This does not only result in an increased storage capacity but in a change in the composition of the stored compounds. Crops in which improvements in
5 yield in seed are especially important are maize, rice, cereals, pea, oilseed rape, sunflower, soybean and legumes. Furthermore, all fruitbearing plants are important for the application of developing a change in the amount and composition of stored carbohydrates. Especially for fruit the composition of stored products gives changes
10 in solidity and firmness, which is especially important in soft fruits like tomato, banana, strawberry, peach, berries and grapes.

In contrast to the effects seen with the expression of TPP, the expression of TPS reduces the ratio of protein/carbohydrate in leaves. This effect is of importance in leafy crops such as fodder grasses and
15 alfalfa. Furthermore, the leaves have a reduced biomass, which can be of importance in amenity grasses, but, more important, they have a relatively increased energy content. This property is especially beneficial for crops as onion, leek and silage maize.

Furthermore, also the viability of the seeds can be influenced
20 by the level of intracellularly available T-6-P.

Combinations of expression of TPP in one part of a plant and TPS in an other part of the plant can synergize to increase the above-described effects. It is also possible to express the genes sequential during development by using specific promoters. Lastly, it is also
25 possible to induce expression of either of the genes involved by placing the coding the sequence under control of an inducible promoter. It is envisaged that combinations of the methods of application as described will be apparent to the person skilled in the art.

30 The invention is further illustrated by the following examples. It is stressed that the Examples show specific embodiments of the inventions, but that it will be clear that variations on these examples and use of other plants or expression systems are covered by the invention.

EXPERIMENTAL**DNA manipulations**

All DNA procedures (DNA isolation from *E.coli*, restriction, ligation, transformation, etc.) are performed according to standard protocols (Sambrook et al. (1989) Molecular Cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, CSH, New York).

Strains

10 In all examples *E.coli* K-12 strain DH5 α is used for cloning. The *Agrobacterium tumefaciens* strains used for plant transformation experiments are EHA 105 and MOG 101 (Hood et al. (1993) Trans. Research 2, 208).

15 **Construction of Agrobacterium strain MOG101**

Construction of Agrobacterium strain MOG101 is described in WO 96/21030.

Cloning of the *E.coli* otsA gene and construction of pMOG799

20 In *E.coli* trehalose phosphate synthase (TPS) is encoded by the *otsA* gene located in the operon *otsBA*. The cloning and sequence determination of the *otsA* gene is described in detail in Example I of WO95/01446, herein incorporated by reference. To effectuate its expression in plant cells, the open reading frame has been linked to
25 the transcriptional regulatory elements of the CaMV 35S RNA promoter, the translational enhancer of the ALMV leader, and the transcriptional terminator of the *nos*-gene, as described in greater detail in Example I of WO95/01446, resulting in pMOG799. A sample of an *E.coli* strain harbouring pMOG799 has been deposited under the Budapest Treaty at the
30 Centraal Bureau voor Schimmelcultures, Oosterstraat 1, P.O. Box 273, 3740 AG Baarn, The Netherlands, on Monday 23 August, 1993: the Accession Number given by the International Depositary Institution is CBS 430.93.

Isolation of a patatin promoter/construction of pMOG546

A patatin promoter fragment is isolated from chromosomal DNA of *Solanum tuberosum* cv. Bintje using the polymerase chain reaction. A set of oligonucleotides, complementary to the sequence of the upstream
 5 region of the λ pat21 patatin gene (Bevan et al. (1986) Nucl. Acids Res. 14, 5564), is synthesized consisting of the following sequences:

5' AAG CTT ATG TTG CCA TAT AGA GTA G 3' PatB33.2 (SEQIDNO:5)

5' GTA GTT GCC ATG GTG CAA ATG TTC 3' PatATG.2 (SEQIDNO:6)

10

These primers are used to PCR amplify a DNA fragment of 1123bp, using chromosomal DNA isolated from potato cv. Bintje as a template. The amplified fragment shows a high degree of similarity to the λ pat21 patatin sequence and is cloned using EcoRI linkers into a pUC18 vector
 15 resulting in plasmid pMOG546.

Construction of pMOG845.

Construction of pMOG845 is described in WO 96/21030.

20 Construction of pVDH318. plastocyanin-TPS

Plasmid pMOG798 (described in WO95/01446) is digested with HindIII and ligated with the oligonucleotide duplex TCV11 and TCV12 (see construction of pMOG845). The resulting vector is digested with PstI and HindIII followed by the insertion of the PotPiII terminator
 25 resulting in pTCV118. Plasmid pTCV118 is digested with SmaI and HindIII yielding a DNA fragment comprising the TPS coding region and the PotPiII terminator. BglII linkers were added and the resulting fragment was inserted in the plant binary expression vector pVDH275 (Fig. 1) digested with BamHI, yielding pVDH318. pVDH275 is a
 30 derivative of pMOG23 (Sijmons et al. (1990), Bio/Technol. 8. 217) harbouring the NPTII selection marker under control of the 35S CaMV promoter and an expression cassette comprising the pea plastocyanin (PC) promoter and nos terminator sequences. The plastocyanin promoter present in pVDH275 has been described by Pwee & Gray (1993) Plant J.
 35 3, 437. This promoter has been transferred to the binary vector using PCR amplification and primers which contain suitable cloning sites.

Cloning of the *E. coli* otsB gene and construction of pMOG1010 (35S CaMV TPP)

A set of oligonucleotides, TPP I (5' CTCAGATCTGGCCACAAA 3') (SEQ ID NO: 56) and TPP II (5' GTGCTCGTCTGCAGGTGC 3') (SEQ ID NO: 57), was synthesized complementary to the sequence of the *E. coli* TPP gene (SEQ ID NO: 3). These primers were used to PCR amplify a DNA fragment of 375bp harbouring the 3' part of the coding region of the *E. coli* TPP gene, introducing a PstI site 10bp down-stream of the stop codon, using pMOG748 (WO 95/01446) as a template. This PCR fragment was digested with BglII and PstI and cloned into pMOG445 (EP 0 449 376 A2 example 7a) and linearized with BglII and PstI. The resulting vector was digested with PstI and HindIII and a PotPIII terminator was inserted (see construction pMOG845). The previous described vector was digested with BglII and HindIII, the resulting 1325 bp fragment was isolated and cloned together with the 5' TPP PCR fragment digested with SmaI and BglII into pUC18 linearized with SmaI and HindIII. The resulting vector was called pTCV124. This vector was linearized with EcoRI and SmaI and used to insert the 35S CaMV promoter (a 850bp EcoRI-'NcoI' (the NcoI site was made blunt by treatment with mungbean nuclease) fragment isolated from pMOG18 containing the 35S CaMV double enhancer promoter). This vector was called pTCV127. From this vector a 2.8kb EcoRI-HindIII fragment was isolated containing the complete 35S TPP expression cassette and cloned in binary vector pMOG800 resulting in vector pMOG1010.

25

Construction of pVDH321, plastocyanin (PC) TPP

The BamHI site of plasmid pTCV124 was removed by BamHI digestion, filling-in and subsequent religation. Subsequent digestion with HindIII and EcoRI yields a DNA fragment comprising the TPP coding region and the PotPIII terminator. BamHI linkers were added and the resulting fragment was inserted in the plant binary expression vector pVDH275 (digested with BamHI) yielding pVDH321.

30

Construction of a patatin TPP expression vector

Similar to the construction of the patatin TPS expression vector (see construction of pMOG845), a patatin TPP expression vector was constructed yielding a binary vector (pMOG1128) which, after
5 transformation, can effectuate expression of TPP in a tuber-specific manner.

Construction of other expression vectors

Similar to the construction of the above mentioned vectors, gene
10 constructs can be made where different promoters are used, in combination with TPS, TPP or trehalase using binary vectors with the NPTII gene or the Hygromycin-resistance gene as selectable marker gene. A description of binary vector pMOG22 harbouring a HPT selection marker is given in Goddijn et al. (1993) Plant J. 4, 863.

15

Triparental matings

The binary vectors are mobilized in triparental matings with the *E.coli* strain HB101 containing plasmid pRK2013 (Ditta et al. (1980) Proc. Natl. Acad. Sci. USA 77, 7347) into *Agrobacterium tumefaciens*
20 strain MOG101 or EHA105 and used for transformation.

Transformation of tobacco (*Nicotiana tabacum* cv. SR1 or cv. Samsun NN)

Tobacco was transformed by cocultivation of plant tissue with *Agrobacterium tumefaciens* strain MOG101 containing the binary vector
25 of interest as described. Transformation was carried out using cocultivation of tobacco leaf disks as described by Horsch et al. (1985) Science 227, 1229. Transgenic plants are regenerated from shoots that grow on selection medium containing kanamycin, rooted and transferred to soil.

30

Transformation of potato

Potato (*Solanum tuberosum* cv. Kardal) was transformed with the *Agrobacterium* strain EHA 105 containing the binary vector of interest. The basic culture medium was MS30R3 medium consisting of MS salts
35 (Murashige and Skoog (1962) Physiol. Plant. 14, 473), R3 vitamins (Ooms et al. (1987) Theor. Appl. Genet. 73, 744), 30 g/l sucrose, 0.5 g/l MES with final pH 5.8 (adjusted with KOH) solidified when necessary with 8 g/l Daichin agar. Tubers of *Solanum tuberosum* cv.

Kardal were peeled and surface sterilized by burning them in 96% ethanol for 5 seconds. The flames were extinguished in sterile water and cut slices of approximately 2 mm thickness. Disks were cut with a bore from the vascular tissue and incubated for 20 minutes in MS30R3 medium containing $1-5 \times 10^8$ bacteria/ml of *Agrobacterium* EHA 105 containing the binary vector. The tuber discs were washed with MS30R3 medium and transferred to solidified postculture medium (PM). PM consisted of M30R3 medium supplemented with 3.5 mg/l zeatin riboside and 0.03 mg/l indole acetic acid (IAA). After two days, discs were transferred to fresh PM medium with 200 mg/l cefotaxim and 100 mg/l vancomycin. Three days later, the tuber discs were transferred to shoot induction medium (SIM) which consisted of PM medium with 250 mg/l carbenicillin and 100 mg/l kanamycin. After 4-8 weeks, shoots emerging from the discs were excised and placed on rooting medium (MS30R3-medium with 100 mg/l cefotaxim, 50 mg/l vancomycin and 50 mg/l kanamycin). The shoots were propagated axenically by meristem cuttings.

Transformation of lettuce

Transformation of lettuce, *Lattuca sativa* cv. Evola was performed according to Curtis et al. (1994) J. Exp. Bot. 45, 1441.

Transformation of sugarbeet

Transformation of sugarbeet, *Beta vulgaris* (maintainer population) was performed according to Fry et al. (1991) Third International Congress of ISPMB, Tucson USA Abstract No. 384, or according to Krens et al. (1996). Plant Sci. 116, 97.

Transformation of *Lycopersicon esculentum*

Tomato transformation was performed according to Van Roekel et al. (1993) Plant Cell Rep. 12, 644.

Transformation of *Arabidopsis*

Transformation of *Arabidopsis thaliana* was carried out either by the method described by Clarke et al. (1992) Plant. Mol. Biol. Rep. 10, 178 or by the method described by Valvekens et al. (1988) Proc. Natl. Acad. Sci. USA, 85, 5536.

Induction of micro-tubers

Stem segments of *in vitro* potato plants harbouring an auxiliary meristem were transferred to micro-tuber inducing medium. Micro-tuber inducing medium contains 1 X MS-salts supplemented with R3 vitamins, 0.5 g/l MES (final pH= 5.8, adjusted with KOH) and solidified with 8 g/l Daichin agar, 60 g/l sucrose and 2.5 mg/l kinetin. After 3 to 5 weeks of growth in the dark at 24°C, micro-tubers were formed.

Isolation of Validamycin A

Validamycin A has been found to be a highly specific inhibitor of trehalases from various sources ranging from (IC₅₀) 10⁻⁶M to 10⁻¹⁰M (Asano et al. (1987) J. Antibiot. 40, 526; Kameda et al. (1987) J. Antibiot. 40, 563). Except for trehalase, it does not significantly inhibit any α - or β -glycohydrolase activity. Validamycin A was isolated from Solacol, a commercial agricultural formulation (Takeda Chem. Indust., Tokyo) as described by Kendall et al. (1990) Phytochemistry 29, 2525. The procedure involves ion-exchange chromatography (QAE-Sephadex A-25 (Pharmacia), bed vol. 10 ml, equilibration buffer 0.2 mM Na-Pi pH 7) from a 3% agricultural formulation of Solacol. Loading 1 ml of Solacol on the column and eluting with water in 7 fractions, practically all Validamycin was recovered in fraction 4. Based on a 100% recovery, using this procedure, the concentration of Validamycin A was adjusted to 1.10⁻³ M in MS-medium, for use in trehalose accumulation tests. Alternatively, Validamycin A and B may be purified directly from *Streptomyces hygroscopicus* var. *limoneus*, as described by Iwasa et al. (1971) J. Antibiot. 24, 119, the content of which is incorporated herein by reference.

Carbohydrate analysis

Carbohydrates were determined quantitatively by anion exchange chromatography with pulsed electrochemical detection. Extracts were prepared by extracting homogenized frozen material with 80% EtOH. After extraction for 15 minutes at room temperature, the soluble fraction is evaporated and dissolved in distilled water. Samples (25 μ l) were analyzed on a Dionex DX-300 liquid chromatograph equipped with a 4 x 250 mm Dionex 35391 carbopac PA-1 column and a 4 x 50 mm Dionex 43096 carbopac PA-1 precolumn. Elution was with 100 mM NaOH at

1 ml/min followed by a NaAc gradient. Sugars were detected with a pulsed electrochemical detector (Dionex, PED). Commercially available carbohydrates (Sigma) were used as a standard.

5 Starch analysis

Starch analysis was performed as described in: Aman et al. (1994) Methods in Carbohydrate Chemistry, Volume X (eds. BeMiller et al.), pp 111-115.

10 Expression analysis

The expression of genes introduced in various plant species was monitored using Northern blot analysis.

Trehalose-6-phosphate phosphatase assay

- 15 TPP was assayed at 37°C by measuring the production of [¹⁴C]trehalose from [¹⁴C]trehalose-6-phosphate (Londesborough and Vuorio (1991) J. of Gen. Microbiol. 137, 323). Crude extracts were prepared in 25 mM Tris, HCl pH 7.4, containing 5.5 mM MgCl₂. Samples were diluted to a protein concentration of 1 mg/ml in extraction buffer containing 1 mg/ml BSA.
- 20 Standard assay mixtures (50 µl final volume) contained 27.5 mM Tris, HCl pH 7.4, 5.5 mM MgCl₂, 1 mg/ml BSA and 0.55 mM T-6-P (specific activity 854 cpm/nmol). Reactions were initiated by the addition of 5µl enzyme and terminated after 1 hour by heating for 5 minutes in boiling water. AG1-X8 (formate) anion-exchange resin (BioRad) was
- 25 added and the reaction mixtures were centrifuged after 20 minutes of equilibration at room temperature. The radioactivity in the supernatant of the samples (400 µl) was measured by liquid scintillation counting.

30 Preparation of plant extracts for hexokinase assays

- Frozen plant material was grinded in liquid nitrogen and homogenized for 30 seconds with extraction buffer (EB: 100mM HEPES pH7.0 (KOH), 1% (w/v) PVP, 5mM MgCl₂, 1.5 mM EDTA, 0.1 %v/v β-MeOH) including Proteinase Inhibitors Complete (Boehringer Mannheim). After
- 35 centrifugation, proteins in the supernatant were precipitated using 80% ammoniumsulphate and dissolved in Tris-HCl pH 7.4 and the extract was dialyzed overnight against 100mM Tris-HCl pH 7.4. Part of the sample was used in the hexokinase assay.

Hexokinase assay

Hexokinase activity was measured in an assay containing 0.1 M Hepes-KOH pH 7.0, 4 mM MgCl₂, 5 mM ATP, 0.2 mM NADP⁺, 10 U/ml Creatine Phosphate Kinase (dissolved in 50% glycerol, 0.1% BSA, 50 mM Hepes pH 7.0), 3.5 mM Creatine Phosphate, 7 U/ml Glucose-6-Phosphate Dehydrogenase and 2 mM Glucose by measuring the increase in OD at 340 nm at 25 °C.

When 2 mM Fructose was used instead of glucose as substrate for the hexokinase reaction, 3.8 U/ml Phosphoglucose Isomerase was included.

Alternatively, a hexokinase assay as described by Gancedo et al. (1977) J. Biol. Chem. 252, 4443 was used.

EXAMPLE 1

Expression of the *E. coli* *otsA* gene (TPS) in tobacco and potato

Transgenic tobacco plants were generated harbouring the *otsA* gene driven by the de35SCaMV promoter (pMOG799) or the plastocyanin promoter (pVDH318).

Transgenic potato plants were generated harbouring the *otsA* gene driven by the potato tuber-specific patatin promoter (pMOG845).

Tobacco leaf discs were transformed with the binary vector pMOG799 using *Agrobacterium tumefaciens*. Transgenic shoots were selected on kanamycin.

Leaves of some soil-grown plants did not fully expand in lateral direction, leading to a lancet-shaped morphology (Fig. 31).

Furthermore, apical dominance was reduced resulting in stunted growth and formation of several axillary shoots. Seven out of thirty-two plants showed severe growth reduction, reaching plant heights of 4-30

cm at the time of flowering (Table 1).

39

Table 1. Trehalose accumulation in leaf samples of *otsA* transgenic tobacco plants and their plant length at the time of flowering.

plant-line	trehalose mg.g ⁻¹ fresh weight	height cm
controls	0.00	60-70
799-1	0.04	ND
799-3	0.02	10
799-5	0.08	4
799-15	0.055	30
799-24	0.02	12
799-26	0.05	25
799-32	0.055	30
799-40	0.11	25

5

ND: not determined

Control plants reached lengths of 60-70 cm at the time of flowering. Less seed was produced by transgenic lines with the stunted growth phenotype. Northern blot analysis confirmed that plants having the stunted growth phenotype expressed the *otsA* gene from *E.coli* (Fig. 2). In control plants no transcript could be detected. The functionality of the introduced gene was proven by carbohydrate analyses of leaf material from 32 transgenic greenhouse-grown tobacco plants, revealing the presence of 0.02 to 0.12 mg.g⁻¹ fresh weight trehalose in plants reduced in length (table 1) indicating that the product of the TPS-catalyzed reaction is dephosphorylated by plant phosphatases. Further proof for the accumulation of trehalose in tobacco was obtained by treating crude extracts with porcine trehalase. Prolonged incubation of a tobacco leaf extract with trehalase resulted in complete degradation of trehalose (data not shown). Trehalose was not detected in control plants or transgenic tobacco plants without an aberrant phenotype.

Table 1a. Primary PC-TPS tobacco transformants

Plant-line	Leaf fw (g)	Leaf area cm ²	No. of branches	Plant height cm	Leaf colour	Axillary shoots	Fw/area g/cm ²	Dry matter %	Dry matter /area g/cm ²
ctrl. 1	8.18	349.37	1		wt		0.023	7.21	0.0017
ctrl. 2	10.5	418.89	1		wt		0.025	9.52	0.0024
ctrl. 3	9.99	373.87	1		wt		0.027	12.91	0.0035
ctrl. 4	9.91	362.92	1		wt		0.027	9.59	0.0026
ctrl. 5	9.82	393.84	1		wt		0.025	11.51	0.0029
average							0.0254	10.148	0.0026
2	8.39	290	2	105	wt		0.029	12.16	0.0035
3	9.34	296	1	123	wt		0.032	12.21	0.0039
4	8.36	254	2	130	wt	many	0.033	10.05	0.0033
6	2.28	106	5	90	wt		0.022	11.40	0.0025
8	5.21	133	4	100	dark	many	0.039	7.49	0.0029
10	8.08	258	2	165	dark	many	0.031	12.25	0.0038
11	2.61	64	12	95	dark	many	0.041	9.20	0.0038
13	2.83	92	1	150	dark	many	0.031	8.48	0.0026
16	5.86	209	3	130	dark	many	0.028	10.58	0.0030
17	5.15	224	2	155	wt		0.023	11.65	0.0027
18	17.2	547	1	133	wt		0.031	10.35	0.0033
19	2.13	63	4	80	dark	many	0.034	11.74	0.0040
20	3.44	113	4	90	wt+Da	many	0.030	8.14	0.0025
21	9.88	246	1	105	dark	many	0.040	8.50	0.0034
22	13.1	409	1	135	wt		0.032	10.68	0.0034
23	2.50	73	6	55	dark	many	0.034	8.80	0.0030
24	8.76	286	2	130	wt		0.031	15.07	0.0046
27	7.91	219	1	124	wt		0.036	14.41	0.0052
28	10.0	269	2	117	dark	many	0.038	8.62	0.0032
29	4.17	142	1	85	dark	many	0.029	10.07	0.0030
30	10.2	343	1	160	wt		0.030	9.56	0.0029
32	1.95	61	3	75	dark	many	0.032	8.21	0.0026
33	2.85	96	5	95	wt+Da	many	0.030	11.23	0.0033
34	8.38	244	1	123	wt		0.034	13.60	0.0047
35	5.59	173	3	126	wt		0.032	14.49	0.0047
36	3.28	84	3	100	dark	many	0.039	11.28	0.0044
37	7.80	222	1	125	wt+Da	many	0.035	11.28	0.0040
39	3.70	131	2	123	wt		0.028	17.84	0.0050
40	2.40	68.5	3	108	dark	many	0.035	9.58	0.0034
average							0.032	11.00	0.0035

Transgenic pVDH318 transgenic tobacco plants developed stunted growth and development of small leaves which were darker green and slightly thicker than control leaves, a phenotype similar to the pMOG799 transgenic plants (table 1a). Further analysis of these leaves showed an increased fresh and dry weight per leaf-area compared to the controls (table 1a and 2). The dark green leaves indicate the presence of more chlorophyll in the transgenic leaves (table 1b). Plants transgenic for pMOG799 (35STPS) and pMOG1177 (PCTPS) were analyzed on soluble carbohydrates, chlorophyll, trehalose and starch (Fig. 32). pMOG1177 is functionally identical to pVDH318.

Table 1b. Chlorophyll content of *N. tabacum* leaves (T_0) transgenic for PC-TPS

Sample	Chlorophyll (mg/g leaf)
control 1	0.59
PC TPS 10-1	0.75
PC TPS 10-2	0.80
PC TPS 11	0.60
PC TPS 13	0.81
PC TPS 16	0.90
PC TPS 19	0.64
PC TPS 37	0.96

Note: light conditions during growth will influence the determined levels of chlorophyll significantly. The calculated amounts of chlorophyll may thus only be compared between plants harvested and analyzed within one experiment!

4.2

Table 2. Fresh weight and dry weight data of leaf material transgenic for plastocyanin-TPS_{E.coli}

N. tabacum cv. Samsun NN transgenic for PC-TPS

	Transgene	Control
Fresh weight (g)	0.83	0.78
Dry weight (g)	0.072	0.079
% dry matter	8.70 %	10.10 %
FW/area	39 (139%)	28 (100%)
DW/area	3.46 (121%)	2.87 (100%)
area (units)	208	275

5

Calculation of the ratio between the length and width of the developing leaves clearly indicate that leaves of plants transgenic for PC-TPS are more lancet-shaped (table 3).

10

Potato *Solanum tuberosum* cv. Kardal tuber discs were transformed with *Agrobacterium tumefaciens* EHA105 harbouring the binary vector pMOG845. Transgenics were obtained with transformation frequencies comparable to empty vector controls. All plants obtained were phenotypically indistinguishable from wild type plants indicating that use of a tissue specific promoter prevents the phenotypes observed in plants where a constitutive promoter drives the TPS gene. Micro-tubers were induced on stem segments of transgenic and wild-type plants cultured on microtuber-inducing medium supplemented with 10^{-3} M Validamycin A. As a control, microtubers were induced on medium without Validamycin A. Microtubers induced on medium with Validamycin A showed elevated levels of trehalose in comparison with microtubers grown on medium without Validamycin A (table 4). The presence of small amounts of trehalose in wild-type plants indicates the presence of a functional trehalose biosynthetic pathway.

25

Table 3. Tobacco plants (cv. Samsun NN) transgenic for pVDH318

Transformant	Length (cm)	Width (cm)	Ratio l/w
control 1	12	8	1.50
control 2	13	8.5	1.53
control 3	12	7.5	1.60
control 4	15	9	1.67
control 5	25	16	1.56
control 6	24	16.5	1.45
control 7	28	20	1.40
control 8	25	16	1.56
control 9	26	19	1.37
control 10	21	15	1.40
1318-28	16	8.5	1.88*
1318-29	11	6.5	1.69
1318-30	19	14	1.36
1318-35	19	12	1.58
1318-39	21	16.5	1.27
1318-40	14	7	2.00*
1318-34	21	13	1.62
1318-36	13.5	7	1.93*
1318-37	17	9	1.89*
1318-4	20.5	12	1.71
1318-23	14	4.5	3.78*
1318-22	27	18	1.50
1318-19	9	4	2.25*
1318-2	27	19	1.42
1318-15	11	5	2.20*
1318-10	20	13	1.54
1318-3	25	18	1.39
1318-21	17	8.5	2.00*
1318-16	20	10	2.00*
1318-6	19	10.5	1.81
1318-20	13	5	2.60*
1318-33	12	5	2.40*
1318-27	23	20	1.15
1318-11	12	5	2.40*
1318-8	18.5	6.5	2.85*
1318-24	27	17	1.59
1318-13	15	7	2.14*
1318-17	24	16	1.50
1318-18	23	16.5	1.39

* typical TPS phenotypes

Ratio l/w average of controls is 1.50

Table 4. Trehalose (% fresh weight)

	+Validamycin A	-Validamycin A
845-2	0.016	-
845-4	-	-
845-8	0.051	-
845-11	0.015	-
845-13	0.011	-
845-22	0.112	-
845-25	0.002	-
845-28	0.109	-
wild-type Kardal	0.001	-

5

EXAMPLE 2

Expression of the *E. coli* *otsB* gene (TPP) in tobacco
 Transgenic tobacco plants were generated harbouring the *otsB* gene driven by the double enhanced 35SCaMV promoter (pMOG1010) and the plastocyanin promoter (pVDH321).

- 10 Tobacco plants (cv. Samsun NN) transformed with pMOG1010 revealed in the greenhouse the development of very large leaves (leaf area increased on average up to approximately 140%) which started to develop chlorosis when fully developed (Fig. 31). Additionally, thicker stems were formed as compared to the controls, in some
- 15 instances leading to bursting of the stems. In some cases, multiple stems were formed (branching) from the base of the plant (table 5). Leaf samples of plants developing large leaves revealed 5-10 times enhanced trehalose-6-phosphate phosphatase activities compared to control plants proving functionality of the gene introduced. The dry
- 20 and fresh weight/cm² of the abnormal large leaves was comparable to control leaves, indicating that the increase in size is due to an increase in dry matter and not to an increased water content. The inflorescence was also affected by the expression of TPP. Plants which had a stunted phenotype, probably caused by the constitutive
- 25 expression of the TPP gene in all plant parts, developed many small flowers which did not fully mature and fell off or necrotized. The development of flowers and seed setting seems to be less affected in plants which were less stunted.

45

Table 5. Tobacco plants transgenic for pMOG1010, de35S CaMV TPP

Line	Height (cm)	Leaf area cm ²	Bleaching (5-severe)	Branch ing	Fw/cm2 (g)	DW/cm2 (g)	Inflor- escence Nozm. /	Stem dia-meter (mm)
1	63	489	5	+	0.096	0.0031	A	13
2	90	472	3	+	0.076	0.0035	A	19
3	103	345	0		0.072	0.0023	N	16
4	90	612	4	+	0.096	0.0039	A	5,6,7,8,14
5	104	618	1	+	0.08	0.0035	N	17
6	110	658	3	+	0.078	0.0035	N/A	19
7	120	427	0		0.074	0.0037	N	18
8	90	472	2	+	0.076	0.0023	A	6,7,18
9	60	354	3	+	0.092	0.0031	N	9,13
10	103	342	0		0.084	0.0025	N	16
11	110	523	1	+	0.076	0.0031	A	18
12	90	533	1	+	0.098	0.0023	N	5,16
13	53	432	4	+	0.084	0.0043	A	5,6,6,14
14	125	335	0		0.086	0.0023	N	17
15	85	251	0		0.094	0.0031	N	14
16	64	352	0	+	0.076	0.0028	A	9,13
17	64	267	0		0.11	0.0018	N	15
18	71	370	2		0.086	0.0032	A	5,7,8,14
19	92	672	4	+	0.076	0.0034	N	16
20								
21	94	517	4	+	0.07	0.0044	N	17
22	96	659	3	+	0.082	0.0031	N	17
23	110	407	0		0.082	0.0042	N	16
24	90	381	0		0.1	0.0034	A	15
25	120	535	0		0.076	0.003	N	16
26	42	511	5		0.08	0.0038	?	15
27	100	468	0		0.086	0.0018	N	17
28	83	583	3		0.072	0.0034	N/A	17
29	27	452	5	+	0.104	0.004	?	7,7,15
30	23	479	4	+	0.076	0.0027	?	6,6,7,9,14
31	103	308	1		0.086	0.0027	N	14
32	48	286	0		0.108	0.002	N	16
33	67	539	5	+	0.102	0.0056	A	18
34	40	311	5	+	0.084	0.0051	A	7,7,12

46

Table 6. Primary PC-TPP tobacco transformants

Plant-line	Leaf fw (g)	Leaf area cm ²	No. of branches	Plant height cm	Leaf colour	Bleaching	Fw/area	Dry matter %	Dry matter /area
ctrl. 1	8.18	349.37					0.023	7.213	
ctrl. 2	10.5	418.89					0.025	9.524	
ctrl. 3	9.99	373.87					0.027	12.913	
ctrl. 4	9.91	362.92					0.027	9.586	
ctrl. 5	9.82	393.84					0.025	11.507	
						average	0.0255	10.149	0.0026
11	11.5	338	3	114	wt		0.0340	6.43	0.0022
12	20.1	742			pale	bleaching	0.0272	9.82	0.0027
14	9.61	345	1	150	wt		0.0279	11.65	0.0032
16	5.99	234	5	54	pale	bleaching	0.0256	12.85	0.0033
17	9.10	314	3	105	wt		0.0290	8.79	0.0025
18	3.78	158	3	75	pale		0.0239	7.67	0.0018
19	2.98	130	1	70	pale		0.0229	10.74	0.0025
20	8.33	296	3	70	pale	bleaching	0.0281	7.56	0.0021
22	11.5	460	1	117	pale	bleaching	0.0251	3.03	0.0008
24	9.42	369	1	155	wt		0.0255	10.62	0.0027
25	15.9	565	1	170	wt		0.0282	9.54	0.0027
26	8.07	343	2	155	wt		0.0235	15.37	0.0036
28	11.7	411	2	65	pale	bleaching	0.0286	6.90	0.0020
29	11.6	420	1	117	pale	bleaching	0.0277	3.53	0.0010
31	8.21	307	2	153	wt		0.0267	12.79	0.0034
32	4.03	175	1	70	pale		0.0230	18.86	0.0043
34	4.81	203	1	107	pale		0.0237	20.58	0.0049
35	7.86	307	3	130	pale		0.0256	11.45	0.0029
36	4.90	206	2	95	pale		0.0238	22.65	0.0054
37	13.9	475	1	135	wt		0.0293	4.82	0.0014
38	16.6	614	1	90	pale	bleaching	0.0271	3.31	0.0009
39	14.9	560	1	112	wt	bleaching	0.0267	6.08	0.0016
40	24.5	843					0.0292	9.80	0.0029
41	8.86	343	1	115	wt		0.0258	2.93	0.0008
42	6.93	289	1		wt		0.0240	3.32	0.0008
43	11.3	433	136	135	wt		0.0261	6.73	0.0018
44	10.0	341	2	135	wt		0.0294	6.49	0.0019
45	9.40	327	2	135	wt		0.0287	8.51	0.0024
46	9.18	284	2	115	wt		0.0323	15.69	0.0051
						average	0.027	9.60	0.0025

wt = wild-type

Tobacco plants (cv. Samsun NN) transformed with pVDH321 revealed in the greenhouse a pattern of development comparable to pMOG1010 transgenic plants (table 6).

- 5 Plants transgenic for pMOG1010 (35S-TPP) and pMOG1124 (PC-TPP) were analyzed on carbohydrates, chlorophyll, trehalose and starch (Fig. 32). For chlorophyll data see also Table 6a.

Table 6a. Chlorophyll content of *N. tabacum* leaves (T_0) transgenic for

10

PC-TPP

Sample	Chlorophyll (mg/g leaf)	Leaf phenotype
control 1	1.56	wild-type
control 2	1.40	wild-type
control 3	1.46	wild-type
control 4	1.56	wild-type
control 5	1.96	wild-type
PC TPP 12	0.79	bleaching
PC TPP 22	0.76	bleaching
PC TPP 25	1.30	wild-type
PC TPP 37	0.86	wild-type
PC TPP 38	0.74	bleaching

Note: light conditions during growth will influence the determined levels of chlorophyll significantly. The calculated amounts of
 15 chlorophyll may thus only be compared between plants harvested and analyzed within one experiment!

EXAMPLE 3**Isolation of gene fragments encoding trehalose-6-phosphate synthases from *Selaginella lepidophylla* and *Helianthus annuus***

5 Comparison of the TPS protein sequences from *E.coli* and *S.cerevisiae* revealed the presence of several conserved regions. These regions were used to design degenerated primers which were tested in PCR amplification reactions using genomic DNA of *E.coli* and yeast as a template. A PCR program was used with a temperature ramp between the
 10 annealing and elongation step to facilitate annealing of the degenerate primers.

PCR amplification was performed using primer sets TPSdeg 1/5 and TPSdeg 2/5 using cDNA of *Selaginella lepidophylla* as a template.

15 Degenerated primers used (IUB code):

TPSdeg1:	GAY ITI ATI TGG RTI CAY GAY TAY CA	(SEQ ID NO:7)
TPSdeg2:	TIG GIT KIT TYY TIC AYA YIC CIT TYC C	(SEQ ID NO:8)
TPSdeg5:	GYI ACI ARR TTC ATI CCR TCI C	(SEQ ID NO:9)

20

PCR fragments of the expected size were cloned and sequenced. Since a large number of homologous sequences were isolated, Southern blot analysis was used to determine which clones hybridized with *Selaginella* genomic DNA. Two clones were isolated, clone 8 of which
 25 the sequence is given in SEQ ID NO: 42 (PCR primer combination 1/5) and clone 43 of which the sequence is given in SEQ ID NO: 44 (PCR primer combination 2/5) which on the level of amino acids revealed regions with a high percentage of identity to the TPS genes from *E.coli* and yeast.

30 One TPS gene fragment was isolated from *Helianthus annuus* (sunflower) using primer combination TPSdeg 2/5 in a PCR amplification with genomic DNA of *H. annuus* as a template. Sequence and Southern blot analysis confirmed the homology with the TPS genes from *E.coli*, yeast and *Selaginella*. Comparison of these sequences with EST sequences
 35 (expressed sequence tags) from various organisms, see Table 6b and SEQ ID NOS 45-53 and 41, indicated the presence of highly homologous genes in rice and *Arabidopsis*, which supports our invention that most plants contain TPS homologous genes (Fig. 3).

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Table 6b.

dbEST ID.	Genbank Accession No.	Organism	Function
35567	D22143	Oryza sativa	TPS
58199	D35348	Caenorhabditis elegans	TPS
60020	D36432	Caenorhabditis elegans	TPS
87366	T36750	Saccharomyces cerevisiae	TPS
35991	D22344	Oryza sativa	TPS
57576	D34725	Caenorhabditis elegans	TPS
298273	H37578	Arabidopsis thaliana	TPS
298289	H37594	Arabidopsis thaliana	TPS
315344	T76390	Arabidopsis thaliana	TPS
315675	T76758	Arabidopsis thaliana	TPS
317475	R65023	Arabidopsis thaliana	TPS
71710	D40048	Oryza sativa	TPS
401677	D67869	Caenorhabditis elegans	TPS
322639	T43451	Arabidopsis thaliana	TPS
76027	D41954	Oryza sativa	TPP
296689	H35994	Arabidopsis thaliana	TPP
297478	H36783	Arabidopsis thaliana	TPP
300237	T21695	Arabidopsis thaliana	TPP
372119	U37923	Oryza sativa	TPP
680701	AA054930	Brugia malayi	trehalase
693476	C12818	Caenorhabditis elegans	trehalase
311652	T21173	Arabidopsis thaliana	TPP
914068	AA273090	Brugia malayi	trehalase
43328	T17578	Saccharomyces cerevisiae	TPP
267495	H07615	Brassica napus	trehalase
317331	R64855	Arabidopsis thaliana	TPP
15008	T00368	Caenorhabditis elegans	trehalase
36717	D23329	Oryza sativa	TPP
71650	D39988	Oryza sativa	TPP
147057	D49134	Oryza sativa	TPP
401537	D67729	Caenorhabditis elegans	trehalase
680728	AA054884	Brugia malayi	trehalase
694414	C13756	Caenorhabditis elegans	trehalase
871371	AA231986	Brugia malayi	trehalase
894468	AA253544	Brugia malayi	trehalase
86985	T36369	Saccharomyces cerevisiae	TPP

EXAMPLE 4**Isolation of plant TPS and TPP genes from *Nicotiana tabacum***

Fragments of plant TPS- and TPP-encoding cDNA were isolated using PCR on cDNA derived from tobacco leaf total RNA preparations. The column

- 5 "nested" in table 7 indicates if a second round of PCR amplification was necessary with primer set 3 and 4 to obtain the corresponding DNA fragment. Primers have been included in the sequence listing (table 7). Subcloning and subsequent sequence analysis of the DNA fragments obtained with the primer sets mentioned revealed substantial homology
- 10 to known TPS genes (Fig. 4 & 5).

Table 7. Amplification of plant derived TPS and TPP cDNAs

TPS-cDNA	primer 1	primer 2	nes- ted	primer 3	primer 4
"825" bp SEQ ID NO 22 & 23	Tre-TPS-14 SEQ ID NO 30	Deg 1 SEQ ID NO 7	No		
"840" bp SEQ ID NO 18 & 19	Tre-TPS-14 SEQ ID NO 30	Tre-TPS-12 SEQ ID NO 31	Yes	Tre-TPS-13 SEQ ID NO 32	Deg 5 SEQ ID NO 9
"630" bp SEQ ID NO 20 & 21	Tre-TPS-14 SEQ ID NO 30	Tre-TPS-12 SEQ ID NO 31	Yes	Deg 2 SEQ ID NO 8	Deg 5 SEQ ID NO 9

TPP-cDNA	primer 1	primer 2	nested
"723" bp SEQ ID NO 16 & 17	Tre-TPP-5 SEQ ID NO 35	Tre-TPP-16 SEQ ID NO 38	No
"543" bp SEQ ID NO 14	Tre-TPP-7 SEQ ID NO 36	Tre-TPP-16 SEQ ID NO 38	No
"447" bp SEQ ID NO 12	Tre-TPP-11 SEQ ID NO 37	Tre-TPP-16 SEQ ID NO 38	No

EXAMPLE 5**Isolation of a bipartite TPS/TPP gene from *Helianthus annuus*
and *Nicotiana tabacum***

Using the sequence information of the TPS gene fragment from sunflower
5 (*Helianthus annuus*), a full length sunflower TPS clone was obtained
using RACE-PCR technology.

Sequence analysis of this full length clone and alignment with TPS2
from yeast (Fig. 6) and TPS and TPP encoding sequences indicated the
isolated clone encodes a TPS/TPP bipartite enzyme (SEQ ID NO 24, 26
10 and 28). The bipartite clone isolated (pMOG1192) was deposited at the
Central Bureau for Strain collections under the rules of the Budapest
treaty with accession number CBS692.97 at April 21, 1997.

Subsequently, we investigated if other plant species also contain
TPS/TPP bipartite clones. A bipartite TPS/TPP cDNA was amplified from
15 tobacco. A DNA product of the expected size (i.e. 1.5 kb) was detected
after PCR with primers TPS deg1/TRE-TPP-16 and nested with TPS
deg2/TRE-TPP-15 (SEQ ID NO: 33). An identical band appeared with PCR
with TPS deg1/TRE-TPP-6 (SEQ ID NO: 34) and nested with TPS deg2/TRE-
TPP-15. The latter fragment was shown to hybridize to the sunflower
20 bipartite cDNA in a Southern blot experiment. Additionally, using
computer database searches, an *Arabidopsis* bipartite clone was
identified (SEQ ID NO: 39)

EXAMPLE 6**25 Expression of plant derived TPS genes in plants**

Further proof for the function of the TPS genes from sunflower and
Selaginella lepidophylla was obtained by isolating their corresponding
full-length cDNA clones and subsequent expression of these clones in
plants under control of the 35S CaMV promoter. Accumulation of
30 trehalose by expression of the *Selaginella* enzyme has been reported by
Zentella and Iturriaga (1996) (Plant Physiol. 111, Abstract 88).

EXAMPLE 7**Genes encoding TPS and TPP from monocot species**

35 A computer search in Genbank sequences revealed the presence of
several rice EST-sequences homologous to TPS1 and TPS2 from yeast
(Fig. 7) which are included in the sequence listing (SEQ ID NO:
41, 51, 52 and 53).

EXAMPLE 8**Isolation human TPS gene**

A TPS gene was isolated from human cDNA. A PCR reaction was performed on human cDNA using the degenerated TPS primers deg2 and deg5. This
5 led to the expected TPS fragment of 0.6 kb. Sequence analysis (SEQ ID NO.10) and comparison with the TPSyeast sequence indicated that isolated sequence encodes a homologous TPS protein (Fig. 8).

EXAMPLE 9

10 **Inhibition of endogenous TPS expression by anti-sense inhibition**

The expression of endogenous TPS genes can be inhibited by the anti-sense expression of a homologous TPS gene under control of promoter sequences which drive the expression of such an anti-sense TPS gene in
15 cells or tissue where the inhibition is desired. For this approach, it is preferred to use a fully identical sequence to the TPS gene which has to be suppressed although it is not necessary to express the entire coding region in an anti-sense expression vector. Fragments of such a coding region have also shown to be functional in the anti-
20 sense inhibition of gene-expression. Alternatively, heterologous genes can be used for the anti-sense approach when these are sufficiently homologous to the endogenous gene.

Binary vectors similar to pMOG845 and pMOG1010 can be used ensuring that the coding regions of the introduced genes which are to be
25 suppressed are introduced in the reverse orientation. All promoters which are suitable to drive expression of genes in target tissues are also suitable for the anti-sense expression of genes.

EXAMPLE 10

30 **Inhibition of endogenous TPP expression by anti-sense inhibition**

Similar to the construction of vectors which can be used to drive anti-sense expression of tps in cells and tissues (Example 9), vectors can be constructed which drive the anti-sense expression of TPP genes.

EXAMPLE 11**Trehalose accumulation in wild-type tobacco and potato plants grown on Validamycin A**

Evidence for the presence of a trehalose biosynthesis pathway in tobacco was obtained by culturing wild-type plants in the presence of 10^{-3}M of the trehalase inhibitor Validamycin A. The treated plants accumulated very small amounts of trehalose, up to 0.0021% (fw). Trehalose accumulation was never detected in any control plants cultured without inhibitor. Similar data were obtained with wild-type microtubers cultured in the presence of Validamycin A. Ten out of seventeen lines accumulated on average 0.001% trehalose (fw) (table 4). No trehalose was observed in microtubers which were induced on medium without Validamycin A.

15

EXAMPLE 12**Trehalose accumulation in potato plants transgenic for as-trehalase**

Further proof for the presence of endogenous trehalose biosynthesis genes was obtained by transforming wild-type potato plants with a 35S CaMV anti-sense trehalase construct (SEQ ID NO:54 and 55, pMOG1027; described in WO 96/21030). A potato shoot transgenic for pMOG1027 showed to accumulate trehalose up to 0.008% on a fresh weight basis. The identity of the trehalose peak observed was confirmed by specifically breaking down the accumulated trehalose with the enzyme trehalase. Tubers of some pMOG1027 transgenic lines showed to accumulate small amounts of trehalose (Fig. 9)

EXAMPLE 13**Inhibition of plant hexokinase activity by trehalose-6-phosphate**

To demonstrate the regulatory effect of trehalose-6-phosphate on hexokinase activity, plant extracts were prepared and tested for hexokinase activity in the absence and presence of trehalose-6-phosphate.

• Potato tuber extracts were assayed using fructose (Fig. 10, Fig. 11) and glucose (Fig. 11) as substrate. The potato tuber assay using 1 mM T-6-P and fructose as substrate was performed according to Gancedo et al. (1997) J. Biol. Chem. 252, 4443. The following assays on tobacco, rice and maize were performed according to the assay described in the

section experimental.

- Tobacco leaf extracts were assayed using fructose (Fig. 12) and glucose (Fig. 12, Fig 13) as substrate.
- Rice leaf extracts were assayed using fructose and glucose (Fig. 14) as substrate.
- Maize leaf extracts were assayed using fructose and glucose (Fig. 15) as substrate.

EXAMPLE 14

10 Inhibition of hexokinase activity in animal cell cultures by trehalose-6-phosphate

To demonstrate the regulation of hexokinase activity in animal cells, total cell extracts were prepared from mouse hybridoma cell cultures. A hexokinase assay was performed using glucose or fructose as

- 15 substrate under conditions as described by Gancedo et al. (see above). Mouse hybridoma cells were subjected to osmotic shock by exposing a cell pellet to 20% sucrose, followed by distilled water. This crude protein extract was used in the hexokinase assay (50 μ l extract corresponding to ca.200 μ g protein).

20

Table 8. Inhibition of animal hexokinase activity by T-6-P

Substrate	Concentra- tion (mM)	T6P (mM)	V ₀ (ODU/min)	V ₁ (ODU/min)	Inhibi- tion (%)
Glucose	2	0.83	0.0204	0.0133	35
Glucose	20	0.83	0.0214	0.0141	35
Glucose	100	0.83	0.0188	0.0125	34
Fructose	20	0.23	0.0207	0.0205	1
Fructose	20	0.43	0.0267	0.0197	26
Fructose	20	0.83	0.0234	0.0151	35
Fructose	20	1.67	0.0246	0.0133	46

The data obtained clearly showed that hexokinase activity in mouse cell extracts is inhibited by trehalose-6-phosphate. The T-6-P concentration range in which this effect is noted is comparable to what has been observed in crude plant extracts. No difference is noted
5 in the efficiency of hexokinase inhibition by trehalose-6-phosphate using glucose or fructose as substrate for the enzyme.

EXAMPLE 15

Photosynthesis and respiration of TPS and TPP expressing 10 tobacco plants

Using tobacco plants transgenic for 35S-TPP (1010-5), PC-TPS (1318-10 and 1318-37) and wild-type Samsun NN plants, effects of expression of these genes on photosynthesis and respiration were determined in leaves.

15 Measurements were performed in a gas exchange-experimental set-up. Velocities of gas-exchange were calculated on the basis of differences in concentration between ingoing and outgoing air using infra-red gas-analytical equipment. Photosynthesis and respiration were measured
20 from identical leaves. From each transgenic plant, the youngest, fully matured leaf was used (upper-leaf) and a leaf that was 3-4 leaf-"stores" lower (lower-leaf).

Photosynthesis was measured as a function of the photosynthetic active
25 light intensity (PAR) from 0-975 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ (200 Watt m^{-2}), in four-fold at CO_2 -concentrations of 350 vpm and 950 vpm.

Respiration was measured using two different time-scales. Measurements performed during a short dark-period after the photosynthesis
30 experiments are coded RD in table 9. These values reflect instantaneous activity since respiration varies substantially during the dark-period. Therefor, the values for the entire night-period were also summed as shown in table 10 (only measured at 350 vpm CO_2).

Table 9. Rate of photosynthesis and respiration, STD is standard deviation

Upper leaf		350 ppm		950 ppm	
		micromol/m ² /s	STD	micromol/m ² /s	STD
Wild-type	RD	0.0826	0.048	1.016	0.142
	EFF	0.060	0.004	0.087	0.004
	AMAX	11.596	0.588	19.215	0.942
1010-5	RD	0.873	0.060	1.014	0.134
	EFF	0.059	0.002	0.090	0.007
	AMAX	12.083	1.546	18.651	1.941
1318-10	RD	0.974	0.076	1.078	0.108
	EFF	0.064	0.003	0.088	0.008
	AMAX	16.261	2.538	24.154	1.854
1318-37	RD	1.067	0.140	1.204	0.116
	EFF	0.061	0.002	0.084	0.011
	AMAX	16.818	2.368	25.174	2.093
Lower leaf					
Wild-type	RD	0.0438	0.079	0.526	0.112
	EFF	0.068	0.002	0.085	0.004
	AMAX	6.529	1.271	11.489	1.841
1010-5	RD	0.455	0.068	0.562	0.118
	EFF	0.064	0.002	0.085	0.006
	AMAX	8.527	0.770	13.181	1.038
1318-10	RD	0.690	0.057	0.828	0.086
	EFF	0.064	0.008	0.085	0.005
	AMAX	11.562	1.778	20.031	1.826
1318-37	RD	0.767	0.033	0.918	0.099
	EFF	0.073	0.006	0.103	0.004
	AMAX	13.467	1.818	19.587	1.681

Table 10. Respiration during 12 hour dark period (mmol CO₂)

STD is standard deviation

	Upper leaf	STD	Lower leaf	STD
Wild-type	25.17	0.82	13.19	1.98
1010-5	30.29	5.09	13.08	1.52
1318-10	28.37	4.50	20.47	0.87
1318-37	32.53	2.01	17.7	1.03

5

In contrast to the respiration in the upper-leaves, in lower leaves the respiration of TPS transgenic plants is significantly higher than for wild-type and TPP plants (table 10) indicating a higher metabolic activity. The decline in respiration during aging of the leaves is significantly less for TPS transgenic plants.

Also, the photosynthetic characteristics differed significantly between on the one hand TPS transgenic plants and on the other hand TPP transgenic and wild-type control plants. The AMAX values (maximum of photosynthesis at light saturation), efficiency of photosynthesis (EFF) and the respiration velocity during a short dark-period after the photosynthetic measurements (RD) are shown in table 9. On average, the upper TPS leaves had a 35% higher AMAX value compared to the TPP and wild-type leaves. The lower leaves show even a higher increased rate of photosynthesis (88%).

To exclude that differences in light-absorption were causing the different photosynthetic rates, absorption values were measured with a SPAD-502 (Minolta). No significant differences in absorption were measured (table 11).

Table 11. Absorbtion values of transgenic lines

Absorbtion (%)	Upper-leaf	Lower-leaf
Wild-type Samson NN	84	83
1010-5	84	82
1318-10	85	86
1318-37	86	86

5

EXAMPLE 16**Chlorophyll-fluorescence of TPS and TPP expressing tobacco plants**

Using tobacco plants transgenic for 35S-TPP (1010-5), PC-TPS (1318-10 and 1318-37) and wild-type Samsun NN plants, effects of expressing these genes were determined on chlorophyll fluorescence of leaf material. Two characteristics of fluorescence were measured:

- 1) ETE (electron transport efficiency), as a measure for the electron transport velocity and the generation of reducing power, and
- 2) Non-photochemical quenching, a measure for energy-dissipation caused by the accumulation of assimilates.

Plants were grown in a greenhouse with additional light of 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (04:00 - 20:00 hours). Day/night $T=21^{\circ}\text{C}/18^{\circ}\text{C}$; R.H. $\pm 75\%$. During a night-period preceding the measurements (duration 16 hours), two plants of each genotype were transferred to the dark and two plants to the light ($\pm 430 \mu\text{mol m}^{-2}\text{s}^{-1}$, 20°C , R.H. 70%). The youngest fully matured leaf was measured. The photochemical efficiency of PSII (photosystem II) and the "non-photochemical quenching" parameters were determined as a function of increasing, light intensity. At each light intensity, a 300 sec. stabilisation time was taken. Measurements were performed at 5, 38, 236, 422 and 784 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PAR with a frequency of 3 light-flashes min^{-1} , 350 ppm CO_2 and 20% O_2 . Experiments were replicated using identical plants, reversing the pretreatment from dark to light and vice versa. The fluorescence characteristics are depicted in Fig. 16.

59

The decrease in electron-transport efficiency (ETE) was comparable between TPP and wild-type plants. TPS plants clearly responded less to a increase of light intensity. This difference was most clear in the light pretreatment. These observations are in agreement with the "non-photochemical " quenching data. TPS plants clearly responded less to the additional supply of assimilates by light compared to TPP and wild-type plants. In the case of TPS plants, the negative regulation of accumulating assimilates on photosynthesis was significantly reduced.

10

EXAMPLE 17

**Export and allocation of assimilates in TPS and RPP
expressing tobacco plants**

Using tobacco plants transgenic for 35S-TPP (1010-5) and PC-TPS (1318-
15 37),

- 1) the export of carbon-assimilates from a fully grown leaf
(indicating "relative source activity", Koch (1996) Annu. Rev. Plant
Physiol. Plant. Mol. Biol. 47, 509 and
- 2) the net accumulation of photo-assimilates in sinks ("relative sink
20 activity"), during a light and a dark-period, were determined.

Developmental stage of the plants: flowerbuds just visible. Labelling
technique used: Steady-state high abundance ^{13}C -labelling of
photosynthetic products (De Visser et al. (1997) Plant Cell Environ
25 20, 37). Of both genotypes, 8 plants, using a fully grown leaf, were
labelled with 5.1 atom% $^{13}\text{CO}_2$ during a light-period (10 hours), when
appropriate followed by a dark-period (14 hours). After labelling,
plants were split in: 1) shoot-tip, 2) young growing leaf, 3) young
fully developed leaf (above the leaf being labelled), 4) young stem
30 (above the leaf being labelled), 5) labelled leaf, 6) petiole and base
of labelled leaf, 7) old, senescing leaf, 8) other and oldest leaves
lower than the labelled leaf, 9) stem lower than the labelled leaf,
10) root-tips. Number, fresh and dry weight and ^{13}C percentage (atom %
 ^{13}C) of carbon were determined. Next to general parameters as biomass,
35 dry matter and number of leaves, calculated were: 1) Export of C out
of the labelled leaf; 2) the relative contribution of imported C in
plant parts; 3) the absolute amount of imported C in plant parts; 4)
the relative distribution of imported C during a light period and a
complete light and dark-period.

The biomass above soil of the TPP transgenics was 27% larger compared to the TPS transgenics ($P < 0.001$); also the root-system of the TPP transgenics were better developed. The TPP plants revealed a significant altered dry matter distribution, +39% leaf and +10% stem biomass compared to TPS plants. TPS plants had a larger number of leaves, but a smaller leaf-area per leaf. Total leaf area per TPS plant was comparable with wild-type ($0.4 \text{ m}^2 \text{ plant}^{-1}$)

- Relative source activity of a fully developed leaf

10 The net export rate of photosynthates out of the labelled leaf is determined by the relative decrease of the % "new C" during the night (for TPP 39% and for TPS 56%) and by the total fixated amount present in the plant using the amount of "new C" in the plant (without the labelled leaf) as a measure. After a light period, TPP leaves exported

15 37% compared to 51% for TPS leaves (table 11). In a following dark-period, this percentage increased to respectively 52% and 81%. Both methods support the conclusion that TPS transgenic plants have a significantly enhanced export rate of photosynthetic products compared to the TPP transgenic plants.

20

- Absolute amount of "new C" in plant parts

Export by TPS transgenics was significantly higher compared to TPP transgenics. Young growing TPS leaves import C stronger compared to young growing TPP leaves.

25

- Relative increase of "new C" in plant parts: sink-strength

The relative contribution of "new C" to the concerning plant part is depicted in Fig. 17. This percentage is a measure for the sink-strength. A significant higher sink-strength was present in the TPS

30 transgenics, especially in the shoot-top, the stem above and beneath the labelled leaf and the petiole of the labelled leaf.

6,

- Table 11. Source activity of a full grown labelled leaf: C accumulation and -export. Nett daily accumulation and export of C-assimilates in labelled leaf and the whole plant (above soil) after steady-state ^{13}C -labelling during a light period (day). N=4: LSD values indicated the smallest significant differences for $P < 0.05$

Time (end of)	Transgene	Source activity grown leaf			
		new C in source leaf (% of total C in leaf)	nett C export during night % of "Day"	new C in source leaf (% of new C in plant)	nett C export to plant (% of total new C)
Day	TPS	17.8	-	48.7	51
	TPP	22.6	-	63.0	37
Day + Night	TPS	7.8	56	16.6	81
	TPP	13.8	39	48.4	52
LSD 0.05		2.4		6.1	

- 10 - Relative distribution, within the plant, of "new C" between the plant parts: relative sink strength

The distribution of fixed carbon between plant organs (Fig. 18) confirmed the above mentioned conclusions. TPS transgenic plants revealed a relative large export of assimilates to the shoot-top, the young growing leaf (day) and even the oldest leaf (without axillary meristems), and to the young and old stem.

EXAMPLE 18: Lettuce

Performance of lettuce plants transgenic for PC-TPS and PC-

TPP

- Constructs used in lettuce transformation experiments: PC-TPS and PC-TPP. PC-TPS transgenics were rescued during regeneration by culturing explants on 60 g/l sucrose. The phenotypes of both TPS and TPP transgenic plants are clearly distinguishable from wild-type controls; TPS transgenic plants have thick, dark-green leaves and TPP transgenic plants have light-green leaves with a smoother leaf-edge when compared to wild-type plants.

The morphology of the leaves, and most prominent the leaf-edges, was clearly affected by the expression of TPS and TPP. Leaves transgenic for PC-TPS were far more "notched" than the PC-TPP transgenic leaves that had a more smooth and round morphology (Fig. 19). Leaf extracts of transgenic lettuce lines were analyzed for sugars and starch (Fig. 20).

EXAMPLE 19: Sugarbeet

Performance of sugarbeet plants transgenic for PC-TPS and PC-TPP

Constructs used in sugarbeet transformation experiments: PC-TPS and PC-TPP. Transformation frequencies obtained with both the TPS and the TPP construct were comparable to controls. The phenotypes of both TPS and TPP transgenic plants were clearly distinguishable from wild-type controls; TPS transgenic plants had thick, dark-green leaves and TPP transgenic plants had light-green coloured leaves with slightly taller petioles when compared to wild-type plants (Fig. 21). Taproot diameter was determined for all plants after ca. 8 weeks of growth in the greenhouse. Some PC-TPS transgenic lines having a leaf size similar to the control plants showed a significant larger diameter of the taproot (Fig. 22). PC-TPP transgenic lines formed a smaller taproot compared to the non-transgenic controls. Leaf extracts of transgenic sugarbeet lines were analyzed for sugars and starch (Fig. 20).

EXAMPLE 20: Arabidopsis

Performance of Arabidopsis plants transgenic for PC-TPS and PC-TPP

Constructs used in Arabidopsis transformation experiments: PC-TPS and PC-TPP. The phenotypes of both TPS and TPP transgenic plants were clearly distinguishable from wild-type controls; TPS transgenic plants had thick, dark-green leaves and TPP transgenic plants had larger, bleaching leaves when compared to wild-type plants. Plants with high levels of TPP expression did not set seed.

EXAMPLE 21: Potato**Performance of *Solanum tuberosum* plants transgenic for TPS and TPP constructs**

- 5 Construct: 35S-TPS pMOG799
Plants transgenic for pMOG799 were grown in the greenhouse and tuber-yield was determined (Fig. 23). The majority of the transgenic plants showed smaller leaf sizes when compared to wild-type controls. Plants with smaller leaf-sizes yielded less tuber-mass compared to control
10 lines (Fig. 25).
- Construct: 35S-TPP pMOG1010 and PC-TPP pMOG1124
Plants transgenic for pMOG 1010 and pMOG1124 were grown in the greenhouse and tuber-yield was determined. Tuber-yield (Fig. 24) was
15 comparable or less than the wild-type control lines (Fig. 25).
- Construct: PC-TPS pMOG1093
Plants transgenic for pMOG1093 were grown in the greenhouse and tuber-yield was determined. A number of transgenic lines having leaves with
20 a size comparable to wild-type (B-C) and that were slightly darker green in colour yielded more tuber-mass compared to control plants (Fig. 26). Plants with leaf sizes smaller (D-G) than control plants yielded less tuber-mass.
- 25 Construct: Pat-TPP pMOG1128
Microtubers were induced *in vitro* on explants of pat-TPP transgenic plants. The average fresh weight biomass of the microtubers formed was substantially lower compared to the control lines
- 30 Construct: Pat-TPS pMOG845
Plants transgenic for pMOG 845 were grown in the greenhouse and tuber-yield was determined. Three Pat-TPS lines produced more tuber-mass compared to control lines (Fig. 27)
- 35 Construct: PC TPS Pat TPS; pMOG1129(845-11/22/28)
Plants expressing PC TPS and Pat-TPS simultaneously were generated by retransforming Pat-TPS lines (resistant against kanamycin) with construct pMOG1129, harbouring a PC TPS construct and a hygromycin resistance marker gene, resulting in genotypes pMOG1129(845-11),

pMOG1129(845-22) and pMOG1129(845-28). Tuber-mass yield varied between almost no yield up to yield comparable or higher than control plants (Fig. 28).

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EXAMPLE 22: Tobacco**Performance of *N. tabacum* plants transgenic for TPS and TPP constructs***Root system*

Tobacco plants transgenic for 35S TPP (pMOG1010) or 35S TPS (pMOG799) were grown in the greenhouse. Root size was determined just before flowering. Lines transgenic for pMOG1010 revealed a significantly smaller/larger root size compared to pMOG799 and non-transgenic wild-type tobacco plants.

15 *Influence of expressing TPS and/or TPP on flowering*

Tobacco plants transgenic for 35S-TPS, PC-TPS, 35S-TPP or PC-TPP were cultured in the greenhouse. Plants expressing high levels of the TPS gene revealed significantly slower growth rates compared to wild-type plants. Flowering and senescence of the lower leaves was delayed in these plants resulting in a stay-green phenotype of the normally senescing leaves. Plants expressing high levels of the TPP gene did not make any flowers or made aberrant, not fully developing flower buds resulting in sterility.

25 *Influence of expressing TPS and/or TPP on seed setting*

Tobacco plants transgenic for 35S-TPS, PC-TPS, 35S-TPP or PC-TPP were cultured in the greenhouse. Plants expressing high levels of the TPP gene revealed poor or no development of flowers and absence of seed-setting.

30

Influence of expressing TPS and/or TPP on seed germination

Tobacco plants transgenic for 35S TPP (pMOG1010) or PC TPP were grown in the greenhouse. Some of the transgenic lines, having low expression levels of the transgene, did flower and set seed. Upon germination of S1 seed, a significantly reduced germination frequency was observed (or germination was absent) compared to S1 seed derived from wild-type plants (table 12).

Table 12. Germination of transgenic 35S-TPP seeds

Seedlot	Bleaching	Rel. [TPPmRNA]	Germination
1010-2	+	15.8	delayed
1010-3	-	5.3	delayed
1010-4	+	4.2	delayed
1010-5	+	5.2	delayed
1010-6	+	3.9	delayed
1010-7	-	2.8	delayed
1010-8	+	6.5	delayed
1010-9	+	4.6	delayed
1010-10	-	1.9	normal
1010-11	-	5.7	normal
1010-12	+	1.4	normal
1010-14	-	0.1	normal
1010-15	-	0.3	normal
1010-18	+	5.6	delayed
1010-20	+	6.4	delayed
1010-21	+	9.5	delayed
1010-22	+	8.8	not
1010-23	-	4.5	normal
1010-24	-	10.2	delayed
1010-25	-	4.7	delayed(less)
1010-27	-	4.8	normal
1010-28	+	22.1	delayed
1010-31	+	9.4	delayed(less)
1010-32	-	0.3	delayed(less)
1010-33	+	14.7	delayed

Influence of expressing TPS and/or TPP on seed yield

Seed-yield was determined for S1 plants transgenic for pMOG1010-5. On average, pMOG1010-5 yielded 4.9 g seed/ plant (n=8) compared to 7.8 g seed/ plant (n=8) for wild-type plants. The "1000-grain" weight is 5 0.06 g for line pMOG1010-5 compared to 0.08 g for wild-type Samsun NN. These data can be explained by a reduced export of carbohydrates from the source leaves, leading to poor development of seed "sink" tissue.

Influence of TPS and TPP expression on leaf morphology

10 Segments of greenhouse grown PC-TPS transgenic, PC-TPP transgenic and non-transgenic control tobacco leaves were fixed, embedded in plastic and coupes were prepared to study cell structures using light-microscopy. Cell structures and morphology of cross-sections of the PC-TPP transgenic plants were comparable to those observed in control 15 plants. Cross-sections of PC-TPS transgenics revealed that the spongy parenchyme cell-layer constituted of 7 layers of cells compared to 3 layers in wild-type and TPP transgenic plants (Fig. 29). This finding agrees with our observation that TPS transgenic plant lines form thicker and more rigid leaves compared to TPP and control plants.

20

EXAMPLE 23**Inhibition of cold-sweetening by the expression of trehalose phosphate synthase**

Transgenic potato plants (*Solanum tuberosum* cv. Kardal) were generated 25 harbouring the TPS gene under control of the potato tuber-specific patatin promoter (pMOG845; Example 1). Transgenic plants and wild-type control plants were grown in the greenhouse and tubers were harvested. Samples of tuber material were taken for sugar analysis directly after harvesting and after 6 months of storage at 4°C. Data resulting from 30 the HPLC-PED analysis are depicted in Fig. 30.

What is clearly shown is that potato plants transgenic for TPS_{E.coli} have a lower amount of total sugar (glucose, fructose and sucrose) accumulating in tubers directly after harvesting. After a storage period of 6 months at 4°C, the increase in soluble sugars is 35 significantly less in the transgenic lines compared to the wild-type control lines.

67

EXAMPLE 24**Improved performance of 35S TPS 35S TPP (pMOG851) transgenic tobacco plants under drought stress**

Transgenic tobacco plants were engineered harbouring both the TPS and TPP gene from *E. coli* under control of the 35S CaMV promoter. The expression of the TPS and TPP genes was verified in the lines obtained using Northern blot and enzyme activity measurements. pMOG851-2 was shown to accumulate 0.008 mg trehalose.g⁻¹ fw and pMOG851-5 accumulated 0.09 mg trehalose.g⁻¹ fw. Expression of both genes had a pronounced effect on plant morphology and growth performance under drought stress. When grown under drought stress imposed by limiting water supply, the two transgenic tobacco lines tested, pMOG851-2 and pMOG851-5, yielded total dry weights that were 28% ($P < 0.01$) and 39% ($P < 0.001$) higher than those of wild-type tobacco. These increases in dry weight were due mainly to increased leaf production: leaf dry weights were up to 85% higher for pMOG851-5 transgenic plants. No significant differences were observed under well-watered conditions.

Drought stress experiments

F1 seeds obtained from self-fertilization of primary transformants pMOG851-2 and pMOG851-5 (Goddijn et al. (1997) Plant Physiol. 113, 181) were used in this study. Seeds were sterilized for 10 minutes in 20% household bleach, rinsed five times in sterile water, and sown on half-strength Murashige and Skoog medium containing 10 g.L⁻¹ sucrose and 100 mg.L⁻¹ kanamycin. Wildtype SR1 seeds were sown on plates without kanamycin. After two weeks seedlings from all lines were transferred to soil (sandy loam), and grown in a growth chamber at 22 °C at approximately 100 µE.m⁻² light intensity, 14h.d⁻¹. All plants were grown in equal amounts of soil, in 3.8 liter pots. The plants were watered daily with half-strength Hoagland's nutrient solution. The seedlings of pMOG851-2 and pMOG851-5 grew somewhat slower than the wildtype seedlings. Since we considered it most important to start the experiments at equal developmental stage, we initiated the drought stress treatments of each line when the seedlings were at equal height (10 cm), at an equal developmental stage (4-leaves), and at equal dry weight (as measured from two additional plants of each line). This meant that the onset of pMOG851-2 treatment was two days later than wildtype, and that of pMOG851-5 seven days later than wildtype. From each line, six plants were subjected to drought stress, while four

were kept under well-watered conditions as controls. The wildtype tobacco plants were droughted by maintaining them around the wilting point: when the lower half of the leaves were wilted, the plants were given so much nutrient solution that the plants temporarily regained

5 turgor. In practice, this meant supplying 50 ml of nutrient solution every three days; the control plants were watered daily to keep them at field capacity. The pMOG851-2 and pMOG851-5 plants were then watered in the exact same way as wildtype, i.e., they were supplied with equal amounts of nutrient solution and after equal time intervals

10 as wildtype. The stem height was measured regularly during the entire study period. All plants were harvested on the same day (32 d after the onset of treatment for the wildtype plants), as harvesting the transgenic plants at a later stage would complicate the comparison of the plant lines. At the time of harvest the total leaf area was

15 measured using a Delta-T Devices leaf area meter (Santa Clara, CA). In addition, the fresh weight and dry weight of the leaves, stems and roots was determined.

A second experiment was done essentially in the same way, to analyze the osmotic potential of the plants. After 35 days of drought stress,

20 samples from the youngest mature leaves were taken at the beginning of the light period (n=3).

Air-drying of detached leaves

The water loss from air-dried detached leaves was measured from

25 well-watered, four-week old pMOG851-2, pMOG851-5 and wildtype plants. Per plant line, five plants were used, and from each plant the two youngest mature leaves were detached and airdried at 25% relative humidity. The fresh weight of each leaf was measured over 32 hours. At the time of the experiment samples were taken from comparable,

30 well-watered leaves, for osmotic potential measurements and determination of soluble sugar contents.

Osmotic potential measurements

Leaf samples for osmotic potential analysis were immediately stored in

35 capped 1 ml syringes and frozen on dry ice. Just before analysis the leaf sap was squeezed into a small vial, mixed, and used to saturate a paper disc. The osmotic potential was then determined in Wescor C52 chambers, using a Wescor HR-33T dew point microvolt meter.

69

Chlorophyll fluorescence

Chlorophyll fluorescence of the wildtype, pMOG851-2 and pMOG851-5 plants was measured for each plant line after 20 days of drought treatment, using a pulse modulation (PAM) fluorometer (Walz, Effeltrich, Germany). Before the measurements, the plants were kept in the dark for two hours, followed by a one-hour light period. Subsequently, the youngest mature leaf was dark-adapted for 20 minutes. At the beginning of each measurement, a small ($0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$ modulated at 1.6 KHz) measuring light beam was turned on, and the minimal fluorescence level (F_0) was measured. The maximal fluorescence level (F_m) was then measured by applying a saturation light pulse of $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$, 800 ms in duration. After another 20 s, when the signal was relaxed to near F_0 , brief saturating pulses of actinic light (800 ms in length, $4000 \mu\text{mol m}^{-2} \text{s}^{-1}$) were given repetitively for 30 s with 2 s dark intervals. The photochemical (q_Q) and non-photochemical (q_E) quenching components were determined from the fluorescence/time curve according to Bolhar-Nordenkamp and Oquist (1993). At the moment of measurement, the leaves in question were not visibly wilted. Statistical data were obtained by one-way analysis of variance using the program Number Cruncher Statistical System (Dr. J.L. Hintze, 865 East 400 North, Kaysville, UT 84037, USA).

Chlorophyll fluorescence analysis of drought-stressed plants showed a higher photochemical quenching (q_Q) and a higher ratio of variable fluorescence over maximal fluorescence (F_v/F_m) in pMOG851-5, indicating a more efficiently working photosynthetic machinery (table 13).

70

Table 13. Chlorophyll fluorescence parameters of wild-type (wt) and trehalose-accumulating (pMOG851-2, pMOG851-5) transgenic tobacco plants. P (probability) values were obtained from ANOVA tests analyzing differences per plant line between plants grown under well-watered (control) or dry conditions, as well as differences between each of the transgenic lines and WT, grown under well-watered or dry conditions. F_m : maximal fluorescence; F_v : variable fluorescence ($F_m - F_0$); q_Q : photochemical quenching; q_E : non-photochemical quenching. F_m , F_v are expressed in arbitrary units (chart mm).

		WT	pMOG851-1	pMOG851-5	8-51-2/WT	815-5
F_m	control	174.4	180.4	175.6	ns	ns
	dry	151.5	155.7	167.8	ns	0.0068
	P (ctrl.dry)	0.0004	0.0000	ns		
F_v	control	134.6	143.3	142.8	ns	ns
	dry	118.4	122.1	135.6	ns	0.0011
	P (ctrl.dry)	0.006	0.0000	ns		
F_v / F_m	control	0.771	0.794	0.813	0.059	0.0052
	dry	0.782	0.784	0.809	ns	0.0016
	P (ctrl.dry)	ns	ns	ns		
q_E	control	15.2	23.8	29.9	0.259	0.0085
	dry	25.4	21.6	23.5	ns	ns
	P (ctrl.dry)	0.048	ns	ns		
q_Q	control	91.3	92.4	90.4	ns	ns
	dry	73.69	78.5	92.75	ns	0.0005
	P (ctrl.dry)	0.005	0.006	ns		

Carbohydrate analysis

At the time of harvest, pMOG851-5 plants contained 0.2 mg.g⁻¹ dry weight trehalose, whereas in pMOG851-2 and wildtype the trehalose levels were below the detection limit, under both stressed and
5 unstressed conditions. The trehalose content in pMOG851-5 plants was comparable in stressed and unstressed plants (0.19 and 0.20 mg. g⁻¹ dry weight, respectively). Under well-watered conditions, the levels of glucose and fructose were twofold higher in pMOG851-5 plants than in wildtype. Leaves of stressed pMOG851-5 plants contained about
10 threefold higher levels of each of the four nonstructural carbohydrates starch, sucrose, glucose and fructose, than leaves of stressed wildtype plants. In pMOG851-2 leaves, carbohydrate levels, like chlorophyll fluorescence values, did not differ significantly from those in wildtype. Stressed plants of all lines contained
15 increased levels of glucose and fructose compared to unstressed plants.

Osmotic potential of drought stressed and control plants

During a second, similar experiment under greenhouse conditions, the
20 transgenic plants showed the same phenotypes as described above, and again the pMOG851-5 plants showed much less reduction in growth under drought stress than pMOG851-2 and wildtype plants. The osmotic potential in leaves of droughted pMOG851-5 plants (-1.77 ± 0.39 Mpa) was significantly lower ($P=0.017$) than in wildtype leaves ($-1.00 \pm$
25 0.08 Mpa); pMOG851-2 showed intermediate values (-1.12 ± 0.05 Mpa). Similarly, under well-watered conditions the osmotic potential of pMOG851-5 plants (-0.79 ± 0.05 Mpa) was significantly lower ($P=0.038$) than that of wildtype leaves (-0.62 ± 0.03 Mpa), with pMOG851-2 having intermediate values (-0.70 ± 0.01 Mpa).

30

Airdrying of detached leaves

Leaves of pMOG851-2, pMOG851-5 and wildtype were detached and their fresh weight was measured over 32 hours of airdrying. Leaves of pMOG851-2 and pMOG851-5 plants lost significantly less water ($P<0.05$)
35 than wildtype leaves: after 32 h leaves of pMOG851-5 and pMOG851-2 had 44% and 41% of their fresh weight left, respectively, compared to 30% for wildtype. At the time of the experiment samples were taken from comparable, well-watered leaves for osmotic potential determination and analysis of trehalose, sucrose, glucose and fructose. The two

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transgenic lines had lower osmotic potentials than wildtype ($P < 0.05$), with pMOG851-5 having the lowest water potential (-0.63 ± 0.03 Mpa), wildtype the highest (-0.51 ± 0.02 Mpa) and pMOG851-2 intermediate (-0.57 ± 0.04 Mpa). The levels of all sugars tested were significantly higher in leaves of pMOG851-5 plants than for wildtype leaves resulting in a threefold higher level of the four sugars combined ($P = 0.002$). pMOG851-2 plants contained twofold higher levels of the four sugars combined ($P = 0.09$). The trehalose levels were 0.24 ± 0.02 mg.g⁻¹ DW in pMOG851-5 plants, and below detection in pMOG851-2 and wildtype.

EXAMPLE 25

Performance of TPS and TPP transgenic lettuce plant lines under drought stress

Primary TPS and TPP transformants and wild-type control plants were subjected to drought-stress. Lines transgenic for TPP reached their wilting point first, then control plants, followed by TPS transgenic plants indicating that TPS transgenic lines, as observed in other plant species, have a clear advantage over the TPP and wild-type plants during drought stress.

EXAMPLE 26

Bolting of lettuce plants is affected in plants transgenic for PC-TPS or PC-TPP

Bolting of lettuce is reduced in plants transgenic for PC-TPP (table 14). Plant lines transgenic for PC-TPS show enhanced bolting compared to wild-type lettuce plants.

Table 14. Bolting of lettuce plants

PC-TPP lines	Total # of plants	1. Normal bolting	2. Reduced bolting	3. Visible inflorescence	4. Possible fasciation	5. Completely vegetative
1A	4					4
2A	3				1	2
3A	2	2				
4A	5	1	1	1	2	
5A	5		1	1		3
7A	1		1			
8A	5	4	1			
9A	5	5				
10A	3		1			2
11A	5			2		3
12A	4					4
Control	5	5				

5

EXAMPLE 27**Performance of tomato plants transgenic for TPS and TPP**

Constructs used in tomato transformation experiments: 35S TPP, PC-TPS, PC-TPS as-trehalase, PC-TPP, E8-TPS, E8-TPP, E8 TPS E8 as-trehalase.

Plants transgenic for the TPP gene driven by the plastocyanin promoter

10 and 35S promoter revealed phenotypes similar to those observed in other plants: bleaching of leaves, reduced formation of flowers or absent flower formation leading to small fruits or absence of fruits.

A small number of 35S-TPP transgenic lines generated extreme large fruits. Those fruits revealed enhanced outgrow of the pericarp. Plants

15 transgenic for the TPS gene driven by the plastocyanin promoter and 35S promoter did not form small lancet shaped leaves. Some severely stunted plants did form small dark-green leaves. Plants transgenic for PC-TPS and PC-as-trehalase did form smaller and darker green leaves as compared to control plants.

20 The colour and leaf-edge of the 35S or PC driven TPS and TPP transgenic plants were clearly distinguishable similar to what is observed in other crops.

Plants harbouring the TPS and TPP gene under control of the fruit-

specific E8 promoter did not show any phenotypical differences compared to wild-type fruits. Plants transgenic for E8 TPS E8 as-trehalase produced aberrant fruits with a yellow skin and incomplete ripening.

5

EXAMPLE 28**Performance of potato plants transgenic for as-trehalase and/or TPS**

- 10 Constructs: 35S as-trehalase (pMOG1027) and 35S as-trehalase Pat TPS (pMOG1027(845-11/22/28)).
- Plants expressing 35S as-trehalase and pat-TPS simultaneously were generated by retransforming pat-TPS lines (resistant against kanamycin) with construct pMOG1027, harbouring the 35S as-trehalase
- 15 construct and a hygromycin resistance marker gene, resulting in genotypes pMOG1027(845-11), pMOG1027(845-22) and pMOG1027(845-28). Microtubers were induced *in vitro* and fresh weight of the microtubers was determined. The average fresh weight yield was increased for transgenic lines harbouring pMOG1027 (pMOG845-11/22/28). The fresh
- 20 weight biomass of microtubers obtained from lines transgenic for pMOG1027 only was slightly higher than wild-type control plants. Resulting plants were grown in the greenhouse and tuber yield was determined (Fig. 33). Lines transgenic for 35S as-trehalase or a combination of 35S as-trehalase and pat-TPS yielded significantly more
- 25 tuber-mass compared to control lines. Starch determination revealed no difference in starch content of tubers produced by plant lines having a higher yield (Fig. 34). A large number of the 1027(845-11/22/28) lines produced tubers above the soil out of the axillary buds of the leaves indicating a profound influence of the constructs used on plant
- 30 development. Plant lines transgenic for 35S as-trehalase only did not form tubers above the soil.

Constructs: Pat as-trehalase (pMOG1028) and Pat as-trehalase Pat TPS (pMOG1028(845-11/22/28))

- 35 Plants expressing Pat as-trehalase and Pat-TPS simultaneously were generated by retransforming Pat-TPS lines (resistant against kanamycin) with construct pMOG1028, harbouring the Pat as-trehalase construct and a hygromycin resistance marker gene, resulting in genotypes pMOG1028(845-11), pMOG1028(845-22) and pMOG1028(845-28).

Plants were grown in the greenhouse and tuber yield was determined (Fig. 35). A number of pMOG1028 transgenic lines yielded significantly more tuber-mass compared to control lines. Individual plants transgenic for both Pat TPS and Pat as-trehalase revealed a varying tuber-yield from almost no yield up to a yield comparable to or higher than the control-lines (Fig. 35).

Construct: PC as-trehalase (pMOG1092)

Plants transgenic for pMOG1092 were grown in the greenhouse and tuber-yield was determined. Several lines formed darker-green leaves compared to controls. Tuber-yield was significantly enhanced compared to non-transgenic plants (Fig. 36).

Construct: PC as-trehalase PC-TPS (pMOG 1130)

Plants transgenic for pMOG 1130 were grown in the greenhouse and tuber-yield was determined. Several transgenic lines developed small dark-green leaves and severely stunted growth indicating that the phenotypic effects observed when plants are transformed with TPS is more severe when the as-trehalase gene is expressed simultaneously (see Example 21). Tuber-mass yield varied between almost no yield up to significantly more yield compared to control plants (Fig. 37).

EXAMPLE 29

Overexpression of a potato trehalase cDNA in *N. tabacum*

Construct: de35S CaMV trehalase (pMOG1078)

Primary tobacco transformants transgenic for pMOG1078 revealed a phenotype different from wild-type tobacco, some transgenics have a dark-green leaf colour and a thicker leaf (the morphology of the leaf is not lancet-shaped) indicating an influence of trehalase gene-expression on plant metabolism. Seeds of selfed primary transformants were sown and selected on kanamycin. The phenotype showed to segregate in a mendelian fashion in the S1 generation.

76

DEPOSITS

The following deposits were made under the Budapest Treaty.

The clones were deposited at the Centraal Bureau voor

Schimmelcultures, Oosterstraat 1, P.O. Box 273, 3740 AG Baarn, The

5 Netherlands on April 21, 1997 and received the following numbers:

	<i>Escherichia coli</i>	DH5alpha/pMOG1192	CBS 692.97
		DH5alpha/pMOG1240	CBS 693.97
		DH5alpha/pMOG1241	CBS 694.97
10		DH5alpha/pMOG1242	CBS 695.97
		DH5alpha/pMOG1243	CBS 696.97
		DH5alpha/pMOG1244	CBS 697.97
		DH5alpha/pMOG1245	CBS 698.97

15 Deposited clones:

	pMOG1192	harbors the <i>Helianthus annuus</i> TPS/TPP bipartite cDNA inserted in the multi-copy vector pGEM-T (Promega).
	pMOG1240	harbors the tobacco TPS "825" bp cDNA fragment inserted in pCRscript (Stratagene).
20	pMOG1241	harbors the tobacco TPS "840" bp cDNA fragment inserted in pGEM-T (Promega).
	pMOG1242	harbors the tobacco TPS "630" bp cDNA fragment inserted in pGEM-T (Promega).
	pMOG1243	harbors the tobacco TPP "543" bp cDNA fragment inserted in
25		pGEM-T (Promega).
	pMOG1244	harbors the tobacco TPP "723" bp cDNA fragment inserted in a pUC18 plasmid.
	pMOG1245	harbors the tobacco TPP "447" bp fragment inserted in
		pGEM-T (Promega).

30

List of relevant pMOG### and pVDH### clones

1. Binary vectors

35	pMOG23	Binary vector (ca. 10 Kb) harboring the NPTII selection marker
	pMOG22	Derivative of pMOG23, the NPTII-gene has been replaced by the HPT-gene which confers resistance to hygromycine
	pVDH 275	Binary vector derived from pMOG23, harbors a plastocyanin promoter- nos terminator expression cassette.

- pMOG402 Derivative of pMOG23, a point-mutation in the NPTII-gene
has been restored, no KpnI restriction site present in the
polylinker
- pMOG800 Derivative of pMOG402 with restored KpnI site in
polylinker

2. TPS / TPP expression constructs

- pMOG 799 35S-TPS-3'nos¹
- pMOG 810 idem with Hyg marker
- 10 pMOG 845 Pat-TPS-3'PotPiII
- pMOG 925 idem with Hyg marker
- pMOG 851 35S-TPS-3'nos 35S-TPP(atg)²
- pMOG 1010 de35S CaMV amv leader TPP(gtg) PotPiII
- pMOG 1142 idem with Hyg marker
- 15 pMOG 1093 Plastocyanin- TPS-3'nos
- pMOG 1129 idem with Hyg marker
- pMOG 1177 Plastocyanin- TPS-3'PotPiII 3'nos
- pVDH 318 Identical to pMOG1177
- Functionally identical to pMOG1093
- 20 pMOG 1124 Plastocyanin- TPP(gtg) 3'PotPiII 3'nos
- pVDH 321 Identical to pMOG1124
- pMOG 1128 Patatin TPP(gtg) 3'PotPiII
- pMOG 1140 E8-TPS-3'nos
- pMOG 1141 E8-TPP(gtg)-3'PotPiII

25

3. Trehalase constructs

- pMOG 1028 Patatin as-trehalase 3'PotPiII, Hygromycin resistance
marker
- pMOG 1078 de35S CaMV amv leader trehalase 3'nos
- 30 pMOG 1090 de35S CaMV amv leader as-trehalase 3'nos
- pMOG 1027 idem with Hyg marker
- pMOG 1092 Plastocyanin- as trehalase-3'nos
- pMOG 1130 Plastocyanin- as trehalase-3'nos Plastocyanin-TPS-3'nos
- pMOG 1153 E8-TPS-3'nos E8-as trehalase-3'PotPiII

35

- 1 All constructs harbour the NPTII selection marker unless noted
otherwise
- 2 Two types of TPP constructs have been used as described in
Goddijn et al. (1997) Plant Physiol.113, 181.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5
- (i) APPLICANT:
- (A) NAME: MOGEN International nv
- (B) STREET: Einsteinweg 97
- (C) CITY: Leiden
- 10 (E) COUNTRY: The Netherlands
- (F) POSTAL CODE (ZIP): 2333 CB
- (G) TELEPHONE: (0)71-5258282
- (H) TELEFAX: (0)71-5221471
- 15 (ii) TITLE OF INVENTION: Regulating metabolism by modifying the level of trehalose-6-phosphate
- (iii) NUMBER OF SEQUENCES: 57
- 20 (iv) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)
- 25 (vi) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: EP 96.201.225.8
- (B) FILING DATE: 03-MAY-1996
- 30 (vi) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: EP 96.202.128.3
- (B) FILING DATE: 26-JUL-1996
- (vi) PRIOR APPLICATION DATA:
- 35 (A) APPLICATION NUMBER: EP 96.202.395.8
- (B) FILING DATE: 29-AUG-1996
- (2) INFORMATION FOR SEQ ID NO: 1:
- 40 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1450 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- 45 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- 50 (ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 21..1450
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

79

	ATAAACTCT CCCCAGGACC ATG ACT ATG AGT CGT TTA GTC GTA GTA TCT	50
	Met Thr Met Ser Arg Leu Val Val Val Ser	
	1 5 10	
5	AAC CGG ATT GCA CCA CCA GAC GAG CAC GCC GCC AGT GCC GGT GGC CTT	98
	Asn Arg Ile Ala Pro Pro Asp Glu His Ala Ala Ser Ala Gly Gly Leu	
	15 20 25	
10	GCC GTT GGC ATA CTG GGG GCA CTG AAA GCC GCA GGC GGA CTG TGG TTT	146
	Ala Val Gly Ile Leu Gly Ala Leu Lys Ala Ala Gly Gly Leu Trp Phe	
	30 35 40	
15	GGC TGG AGT GGT GAA ACA GGG AAT GAG GAT CAG CCG CTA AAA AAG GTG	194
	Gly Trp Ser Gly Glu Thr Gly Asn Glu Asp Gln Pro Leu Lys Lys Val	
	45 50 55	
20	AAA AAA GGT AAC ATT ACG TGG GCC TCT TTT AAC CTC AGC GAA CAG GAC	242
	Lys Lys Gly Asn Ile Thr Trp Ala Ser Phe Asn Leu Ser Glu Gln Asp	
	60 65 70	
25	CTT GAC GAA TAC TAC AAC CAA TTC TCC AAT GCC GTT CTC TGG CCC GCT	290
	Leu Asp Glu Tyr Tyr Asn Gln Phe Ser Asn Ala Val Leu Trp Pro Ala	
	75 80 85 90	
30	TTT CAT TAT CGG CTC GAT CTG GTG CAA TTT CAG CGT CCT GCC TGG GAC	338
	Phe His Tyr Arg Leu Asp Leu Val Gln Phe Gln Arg Pro Ala Trp Asp	
	95 100 105	
35	GGC TAT CTA CGC GTA AAT GCG TTG CTG GCA GAT AAA TTA CTG CCG CTG	386
	Gly Tyr Leu Arg Val Asn Ala Leu Leu Ala Asp Lys Leu Leu Pro Leu	
	110 115 120	
40	TTG CAA GAC GAT GAC ATT ATC TGG ATC CAC GAT TAT CAC CTG TTG CCA	434
	Leu Gln Asp Asp Asp Ile Ile Trp Ile His Asp Tyr His Leu Leu Pro	
	125 130 135	
45	TTT GCG CAT GAA TTA CGC AAA CGG GGA GTG AAT AAT CGC ATT GGT TTC	482
	Phe Ala His Glu Leu Arg Lys Arg Gly Val Asn Asn Arg Ile Gly Phe	
	140 145 150	
50	TTT CTG CAT ATT CCT TTC CCG ACA CCG GAA ATC TTC AAC GCG CTG CCG	530
	Phe Leu His Ile Pro Phe Pro Thr Pro Glu Ile Phe Asn Ala Leu Pro	
	155 160 165 170	
55	ACA TAT GAC ACC TTG CTT GAA CAG CTT TGT GAT TAT GAT TTG CTG GGT	578
	Thr Tyr Asp Thr Leu Leu Glu Gln Leu Cys Asp Tyr Asp Leu Leu Gly	
	175 180 185	
60	TTC CAG ACA GAA AAC GAT CGT CTG GCG TTC CTG GAT TGT CTT TCT AAC	626
	Phe Gln Thr Glu Asn Asp Arg Leu Ala Phe Leu Asp Cys Leu Ser Asn	
	190 195 200	
65	CTG ACC CGC GTC ACG ACA CGT AGC GCA AAA AGC CAT ACA GCC TGG GGC	674
	Leu Thr Arg Val Thr Thr Arg Ser Ala Lys Ser His Thr Ala Trp Gly	
	205 210 215	

50

	AAA GCA TTT CGA ACA GAA GTC TAC CCG ATC GGC ATT GAA CCG AAA GAA	722
	Lys Ala Phe Arg Thr Glu Val Tyr Pro Ile Gly Ile Glu Pro Lys Glu	
	220 225 230	
5	ATA GCC AAA CAG GCT GCC GGG CCA CTG CCG CCA AAA CTG GCG CAA CTT	770
	Ile Ala Lys Gln Ala Ala Gly Pro Leu Pro Pro Lys Leu Ala Gln Leu	
	235 240 245 250	
	AAA GCG GAA CTG AAA AAC GTA CAA AAT ATC TTT TCT GTC GAA CCG CTG	818
10	Lys Ala Glu Leu Lys Asn Val Gln Asn Ile Phe Ser Val Glu Arg Leu	
	255 260 265	
	GAT TAT TCC AAA GGT TTG CCA GAG CGT TTT CTC GCC TAT GAA GCG TTG	866
15	Asp Tyr Ser Lys Gly Leu Pro Glu Arg Phe Leu Ala Tyr Glu Ala Leu	
	270 275 280	
	CTG GAA AAA TAT CCG CAG CAT CAT GGT AAA ATT CGT TAT ACC CAG ATT	914
	Leu Glu Lys Tyr Pro Gln His His Gly Lys Ile Arg Tyr Thr Gln Ile	
	285 290 295	
20	GCA CCA ACG TCG CGT GGT GAT GTG CAA GCC TAT CAG GAT ATT CGT CAT	962
	Ala Pro Thr Ser Arg Gly Asp Val Gln Ala Tyr Gln Asp Ile Arg His	
	300 305 310	
25	CAG CTC GAA AAT GAA GCT GGA CGA ATT AAT GGT AAA TAC GGG CAA TTA	1010
	Gln Leu Glu Asn Glu Ala Gly Arg Ile Asn Gly Lys Tyr Gly Gln Leu	
	315 320 325 330	
	GGC TGG ACG CCG CTT TAT TAT TTG AAT CAG CAT TTT GAC CGT AAA TTA	1058
30	Gly Trp Thr Pro Leu Tyr Tyr Leu Asn Gln His Phe Asp Arg Lys Leu	
	335 340 345	
	CTG ATG AAA ATA TTC CGC TAC TCT GAC GTG GGC TTA GTG ACG CCA CTG	1106
35	Leu Met Lys Ile Phe Arg Tyr Ser Asp Val Gly Leu Val Thr Pro Leu	
	350 355 360	
	CGT GAC GGG ATG AAC CTG GTA GCA AAA GAG TAT GTT GCT GCT CAG GAC	1154
	Arg Asp Gly Met Asn Leu Val Ala Lys Glu Tyr Val Ala Ala Gln Asp	
	365 370 375	
40	CCA GCC AAT CCG GGC GTT CTT GTT CTT TCG CAA TTT GCG GGA GCG GCA	1202
	Pro Ala Asn Pro Gly Val Leu Val Leu Ser Gln Phe Ala Gly Ala Ala	
	380 385 390	
45	AAC GAG TTA ACG TCG GCG TTA ATT GTT AAC CCC TAC GAT CGT GAC GAA	1250
	Asn Glu Leu Thr Ser Ala Leu Ile Val Asn Pro Tyr Asp Arg Asp Glu	
	395 400 405 410	
	GTT GCA GCT GCG CTG GAT CGT GCA TTG ACT ATG TCG CTG GCG GAA CGT	1298
50	Val Ala Ala Ala Leu Asp Arg Ala Leu Thr Met Ser Leu Ala Glu Arg	
	415 420 425	
	ATT TCC CGT CAT GCA GAA ATG CTG GAC GTT ATC GTG AAA AAC GAT ATT	1346
55	Ile Ser Arg His Ala Glu Met Leu Asp Val Ile Val Lys Asn Asp Ile	
	430 435 440	

81

AAC CAC TGG CAG GAG TGC TTC ATT AGC GAC CTA AAG CAG ATA GTT CCG 1394
 Asn His Trp Gln Glu Cys Phe Ile Ser Asp Leu Lys Gln Ile Val Pro
 445 450 455

5 CGA AGC GCG GAA AGC CAG CAG CGC GAT AAA GTT GCT ACC TTT CCA AAG 1442
 Arg Ser Ala Glu Ser Gln Gln Arg Asp Lys Val Ala Thr Phe Pro Lys
 460 465 470

CTC TGC AG 1450

10 Leu Cys 475

(2) INFORMATION FOR SEQ ID NO: 2:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 476 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

25 Met Thr Met Ser Arg Leu Val Val Val Ser Asn Arg Ile Ala Pro Pro
 1 5 10 15

Asp Glu His Ala Ala Ser Ala Gly Gly Leu Ala Val Gly Ile Leu Gly
 20 25 30

30 Ala Leu Lys Ala Ala Gly Gly Leu Trp Phe Gly Trp Ser Gly Glu Thr
 35 40 45

Gly Asn Glu Asp Gln Pro Leu Lys Lys Val Lys Lys Gly Asn Ile Thr
 35 50 55 60

Trp Ala Ser Phe Asn Leu Ser Glu Gln Asp Leu Asp Glu Tyr Tyr Asn
 65 70 75 80

40 Gln Phe Ser Asn Ala Val Leu Trp Pro Ala Phe His Tyr Arg Leu Asp
 85 90 95

Leu Val Gln Phe Gln Arg Pro Ala Trp Asp Gly Tyr Leu Arg Val Asn
 100 105 110

45 Ala Leu Leu Ala Asp Lys Leu Leu Pro Leu Leu Gln Asp Asp Ile
 115 120 125

Ile Trp Ile His Asp Tyr His Leu Leu Pro Phe Ala His Glu Leu Arg
 50 130 135 140

Lys Arg Gly Val Asn Asn Arg Ile Gly Phe Phe Leu His Ile Pro Phe
 145 150 155 160

55 Pro Thr Pro Glu Ile Phe Asn Ala Leu Pro Thr Tyr Asp Thr Leu Leu
 165 170 175

82

	Glu	Gln	Leu	Cys	Asp	Tyr	Asp	Leu	Leu	Gly	Phe	Gln	Thr	Glu	Asn	Asp	
				180					185					190			
5	Arg	Leu	Ala	Phe	Leu	Asp	Cys	Leu	Ser	Asn	Leu	Thr	Arg	Val	Thr	Thr	
			195					200					205				
	Arg	Ser	Ala	Lys	Ser	His	Thr	Ala	Trp	Gly	Lys	Ala	Phe	Arg	Thr	Glu	
		210					215					220					
10	Val	Tyr	Pro	Ile	Gly	Ile	Glu	Pro	Lys	Glu	Ile	Ala	Lys	Gln	Ala	Ala	
	225					230					235					240	
	Gly	Pro	Leu	Pro	Pro	Lys	Leu	Ala	Gln	Leu	Lys	Ala	Glu	Leu	Lys	Asn	
					245					250					255		
15	Val	Gln	Asn	Ile	Phe	Ser	Val	Glu	Arg	Leu	Asp	Tyr	Ser	Lys	Gly	Leu	
			260						265					270			
	Pro	Glu	Arg	Phe	Leu	Ala	Tyr	Glu	Ala	Leu	Leu	Glu	Lys	Tyr	Pro	Gln	
20			275					280					285				
	His	His	Gly	Lys	Ile	Arg	Tyr	Thr	Gln	Ile	Ala	Pro	Thr	Ser	Arg	Gly	
		290				295						300					
25	Asp	Val	Gln	Ala	Tyr	Gln	Asp	Ile	Arg	His	Gln	Leu	Glu	Asn	Glu	Ala	
	305					310					315					320	
	Gly	Arg	Ile	Asn	Gly	Lys	Tyr	Gly	Gln	Leu	Gly	Trp	Thr	Pro	Leu	Tyr	
				325						330					335		
30	Tyr	Leu	Asn	Gln	His	Phe	Asp	Arg	Lys	Leu	Leu	Met	Lys	Ile	Phe	Arg	
				340					345					350			
	Tyr	Ser	Asp	Val	Gly	Leu	Val	Thr	Pro	Leu	Arg	Asp	Gly	Met	Asn	Leu	
35			355					360					365				
	Val	Ala	Lys	Glu	Tyr	Val	Ala	Ala	Gln	Asp	Pro	Ala	Asn	Pro	Gly	Val	
		370					375					380					
40	Leu	Val	Leu	Ser	Gln	Phe	Ala	Gly	Ala	Ala	Asn	Glu	Leu	Thr	Ser	Ala	
	385					390					395					400	
	Leu	Ile	Val	Asn	Pro	Tyr	Asp	Arg	Asp	Glu	Val	Ala	Ala	Ala	Leu	Asp	
				405						410					415		
45	Arg	Ala	Leu	Thr	Met	Ser	Leu	Ala	Glu	Arg	Ile	Ser	Arg	His	Ala	Glu	
				420					425					430			
	Met	Leu	Asp	Val	Ile	Val	Lys	Asn	Asp	Ile	Asn	His	Trp	Gln	Glu	Cys	
50			435					440					445				
	Phe	Ile	Ser	Asp	Leu	Lys	Gln	Ile	Val	Pro	Arg	Ser	Ala	Glu	Ser	Gln	
		450					455					460					
55	Gln	Arg	Asp	Lys	Val	Ala	Thr	Phe	Pro	Lys	Leu	Cys					
	465					470					475						

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 835 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- 15 (A) NAME/KEY: CDS
 (B) LOCATION: 18..818

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

20	ATAAACTCT CCCC GGG ATG ACA GAA CCG TTA ACC GAA ACC CCT GAA CTA	50
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	1 5 10	
25	TCC GCG AAA TAT GCC TGG TTT TTT GAT CTT GAT GGA ACG CTG GCG GAA	98
	Ser Ala Lys Tyr Ala Trp Phe Phe Asp Leu Asp Gly Thr Leu Ala Glu	
	15 20 25	
30	ATC AAA CCG CAT CCC GAT CAG GTC GTC GTG CCT GAC AAT ATT CTG CAA	146
	Ile Lys Pro His Pro Asp Gln Val Val Val Pro Asp Asn Ile Leu Gln	
	30 35 40	
35	GGA CTA CAG CTA CTG GCA ACC GCA AGT GAT GGT GCA TTG GCA TTG ATA	194
	Gly Leu Gln Leu Leu Ala Thr Ala Ser Asp Gly Ala Leu Ala Leu Ile	
	45 50 55	
40	TCA GGG CGC TCA ATG GTG GAG CTT GAC GCA CTG GCA AAA CCT TAT CGC	242
	Ser Gly Arg Ser Met Val Glu Leu Asp Ala Leu Ala Lys Pro Tyr Arg	
	60 65 70 75	
45	TTC CCG TTA GCG GGC GTG CAT GGG GCG GAG CGC CGT GAC ATC AAT GGT	290
	Phe Pro Leu Ala Gly Val His Gly Ala Glu Arg Arg Asp Ile Asn Gly	
	80 85 90	
50	AAA ACA CAT ATC GTT CAT CTG CCG GAT GCG ATT GCG CGT GAT ATT AGC	338
	Lys Thr His Ile Val His Leu Pro Asp Ala Ile Ala Arg Asp Ile Ser	
	95 100 105	
55	GTG CAA CTG CAT ACA GTC ATC GCT CAG TAT CCC GGC GCG GAG CTG GAG	386
	Val Gln Leu His Thr Val Ile Ala Gln Tyr Pro Gly Ala Glu Leu Glu	
	110 115 120	
60	GCG AAA GGG ATG GCT TTT GCG CTG CAT TAT CGT CAG GCT CCG CAG CAT	434
	Ala Lys Gly Met Ala Phe Ala Leu His Tyr Arg Gln Ala Pro Gln His	
	125 130 135	

84

GAA GAC GCA TTA ATG ACA TTA GCG CAA CGT ATT ACT CAG ATC TGG CCA 482
 Glu Asp Ala Leu Met Thr Leu Ala Gln Arg Ile Thr Gln Ile Trp Pro
 140 145 150 155
 5 CAA ATG GCG TTA CAG CAG GGA AAG TGT GTT GTC GAG ATC AAA CCG AGA 530
 Gln Met Ala Leu Gln Gln Gly Lys Cys Val Val Glu Ile Lys Pro Arg
 160 165 170
 GGT ACC AGT AAA GGT GAG GCA ATT GCA GCT TTT ATG CAG GAA GCT CCC 578
 10 Gly Thr Ser Lys Gly Glu Ala Ile Ala Ala Phe Met Gln Glu Ala Pro
 175 180 185
 TTT ATC GGG CGA ACG CCC GTA TTT CTG GGC GAT GAT TTA ACC GAT GAA 626
 15 Phe Ile Gly Arg Thr Pro Val Phe Leu Gly Asp Asp Leu Thr Asp Glu
 190 195 200
 TCT GGC TTC GCA GTC GTT AAC CGA CTG GGC GGA ATG TCA GTA AAA ATT 674
 Ser Gly Phe Ala Val Val Asn Arg Leu Gly Gly Met Ser Val Lys Ile
 205 210 215
 20 GGC ACA GGT GCA ACT CAG GCA TCA TGG CGA CTG GCG GGT GTG CCG GAT 722
 Gly Thr Gly Ala Thr Gln Ala Ser Trp Arg Leu Ala Gly Val Pro Asp
 220 225 230 235
 25 GTC TGG AGC TGG CTT GAA ATG ATA ACC ACC GCA TTA CAA CAA AAA AGA 770
 Val Trp Ser Trp Leu Glu Met Ile Thr Thr Ala Leu Gln Gln Lys Arg
 240 245 250
 GAA AAT AAC AGG AGT GAT GAC TAT GAG TCG TTT AGT CGT AGT ATC TAA 818
 30 Glu Asn Asn Arg Ser Asp Asp Tyr Glu Ser Phe Ser Arg Ser Ile *
 255 260 265
 CCGGATTGCA CCTGCAG
 835
 35 270

(2) INFORMATION FOR SEQ ID NO: 4:

40

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 272 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

45

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

50 Met Thr Glu Pro Leu Thr Glu Thr Pro Glu Leu Ser Ala Lys Tyr Ala
 1 5 10 15
 Trp Phe Phe Asp Leu Asp Gly Thr Leu Ala Glu Ile Lys Pro His Pro
 20 25 30
 55

85

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      Asp Gln Val Val Val Pro Asp Asn Ile Leu Gln Gly Leu Gln Leu Leu
      35                                40                                45

5      Ala Thr Ala Ser Asp Gly Ala Leu Ala Leu Ile Ser Gly Arg Ser Met
      50                                55                                60

      Val Glu Leu Asp Ala Leu Ala Lys Pro Tyr Arg Phe Pro Leu Ala Gly
      65                                70                                75                                80

10     Val His Gly Ala Glu Arg Arg Asp Ile Asn Gly Lys Thr His Ile Val
      85                                90                                95

      His Leu Pro Asp Ala Ile Ala Arg Asp Ile Ser Val Gln Leu His Thr
      100                               105                               110

15     Val Ile Ala Gln Tyr Pro Gly Ala Glu Leu Glu Ala Lys Gly Met Ala
      115                               120                               125

      Phe Ala Leu His Tyr Arg Gln Ala Pro Gln His Glu Asp Ala Leu Met
      130                               135                               140

      Thr Leu Ala Gln Arg Ile Thr Gln Ile Trp Pro Gln Met Ala Leu Gln
      145                               150                               155                               160

25     Gln Gly Lys Cys Val Val Glu Ile Lys Pro Arg Gly Thr Ser Lys Gly
      165                               170                               175

      Glu Ala Ile Ala Ala Phe Met Gln Glu Ala Pro Phe Ile Gly Arg Thr
      180                               185                               190

30     Pro Val Phe Leu Gly Asp Asp Leu Thr Asp Glu Ser Gly Phe Ala Val
      195                               200                               205

      Val Asn Arg Leu Gly Gly Met Ser Val Lys Ile Gly Thr Gly Ala Thr
      210                               215                               220

      Gln Ala Ser Trp Arg Leu Ala Gly Val Pro Asp Val Trp Ser Trp Leu
      225                               230                               235                               240

40     Glu Met Ile Thr Thr Ala Leu Gln Gln Lys Arg Glu Asn Asn Arg Ser
      245                               250                               255

      Asp Asp Tyr Glu Ser Phe Ser Arg Ser Ile *
      260                               265

```

45

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
- 50 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 55 (ii) MOLECULE TYPE: cDNA

86

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

5 AAGCTTATGT TGCCATATAG AGTAGAT

27

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

20 GTAGTTGCCA TGGTGCAAAT GTTCATATG

29

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35 (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

40 GAYITIATIT GGRTICAYGA YTAYCA

26

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

87

TIGGITKITT YYTICAYAYI CCITTYCC

28

(2) INFORMATION FOR SEQ ID NO: 9:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 15 (iii) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GYIACIARRT TCATICCRTC IC

22

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 743 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- 35 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..743
 (D) OTHER INFORMATION: /partial

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

45 GAC GTG ATG TGG ATG CAC GAC TAC CAT TTG ATG GTG TTG CCT ACG TTC 48
 Asp Val Met Trp Met His Asp Tyr His Leu Met Val Leu Pro Thr Phe
 1 5 10 15

50 TTG AGG AGG CGG TTC AAT CGT TTG AGA ATG GGG TTT TTC CTT CAC AGT 96
 Leu Arg Arg Arg Phe Asn Arg Leu Arg Met Gly Phe Phe Leu His Ser
 20 25 30

CCA TTT CCC TCA TCT GAG ATT TAC AGG ACA CTT CCT GTT AGA GAG GAA 144
 Pro Phe Pro Ser Ser Glu Ile Tyr Arg Thr Leu Pro Val Arg Glu Glu
 35 40 45

	ATA	CTC	AAG	GCT	TTG	CTC	TGT	GCT	GAC	ATT	GTT	GGA	TTC	CAC	ACT	TTT	192
	Ile	Leu	Lys	Ala	Leu	Leu	Cys	Ala	Asp	Ile	Val	Gly	Phe	His	Thr	Phe	
	50						55					60					
5	GAC	TAC	GCG	AGA	CAC	TTC	CTC	TCT	TGT	TGC	AGT	CGG	ATG	TTG	GGT	TTA	240
	Asp	Tyr	Ala	Arg	His	Phe	Leu	Ser	Cys	Cys	Ser	Arg	Met	Leu	Gly	Leu	
	65					70					75					80	
	GAG	TAT	CAG	TCT	AAA	AGA	GGT	TAT	ATA	GGG	TTA	GAA	TAC	TAT	GGA	CGG	
10	Glu	Tyr	Gln	Ser	Lys	Arg	Gly	Tyr	Ile	Gly	Leu	Glu	Tyr	Tyr	Gly	Arg	288
					85					90					95		
	ACA	GTA	GGC	ATC	AAG	ATT	ATG	CCC	GTC	GGG	ATA	CAT	ATG	GGT	CAT	ATT	
15	Thr	Val	Gly	Ile	Lys	Ile	Met	Pro	Val	Gly	Ile	His	Met	Gly	His	Ile	336
				100					105					110			
	GAG	TCC	ATG	AAG	AAA	CTT	GCA	GCG	AAA	GAG	TTG	ATG	CTT	AAG	GCG	CTA	
20	Glu	Ser	Met	Lys	Lys	Leu	Ala	Ala	Lys	Glu	Leu	Met	Leu	Lys	Ala	Leu	384
			115					120					125				
	AAG	CAG	CAA	TTT	GAA	GGG	AAA	ACT	GTG	TTG	CTT	GGT	GCC	GAT	GAC	CTG	
	Lys	Gln	Gln	Phe	Glu	Gly	Lys	Thr	Val	Leu	Leu	Gly	Ala	Asp	Asp	Leu	432
			130				135					140					
25	GAT	ATT	TTC	AAA	GGT	ATA	AAC	TTA	AAG	CTT	CTA	GCT	ATG	GAA	CAG	ATG	480
	Asp	Ile	Phe	Lys	Gly	Ile	Asn	Leu	Lys	Leu	Leu	Ala	Met	Glu	Gln	Met	
	145					150					155					160	
	CTC	AAA	CAG	CAC	CCC	AAG	TGG	CAA	GGG	CAG	GCT	GTG	TTG	GTC	CAG	ATT	
30	Leu	Lys	Gln	His	Pro	Lys	Trp	Gln	Gly	Gln	Ala	Val	Leu	Val	Gln	Ile	528
					165					170					175		
	GCA	AAT	CCT	ACG	AGG	GGT	AAA	GGA	GTA	GAT	TTT	GAG	GAA	ATA	CAG	GCT	
35	Ala	Asn	Pro	Thr	Arg	Gly	Lys	Gly	Val	Asp	Phe	Glu	Glu	Ile	Gln	Ala	576
				180					185					190			
	GAG	ATA	TCG	GAA	AGC	TGT	AAG	AGA	ATC	AAT	AAG	CAA	TTC	GGC	AAG	CCT	
40	Glu	Ile	Ser	Glu	Ser	Cys	Lys	Arg	Ile	Asn	Lys	Gln	Phe	Gly	Lys	Pro	624
			195					200					205				
	GGA	TAT	GAG	CCT	ATA	GTT	TAT	ATT	GAT	AGG	CCC	GTG	TCA	AGC	AGT	GAA	
	Gly	Tyr	Glu	Pro	Ile	Val	Tyr	Ile	Asp	Arg	Pro	Val	Ser	Ser	Ser	Glu	672
			210				215					220					
45	CGC	ATG	GCA	TAT	TAC	AGT	ATT	GCA	GAA	TGT	GTT	GTT	GTC	ACG	GCT	GTG	720
	Arg	Met	Ala	Tyr	Tyr	Ser	Ile	Ala	Glu	Cys	Val	Val	Val	Thr	Ala	Val	
	225					230					235					240	
	AGC	GAC	GGC	ATG	AAC	TTC	GTC	TC									
50	Ser	Asp	Gly	Met	Asn	Phe	Val										743
					245												

89

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 247 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```

Asp Val Met Trp Met His Asp Tyr His Leu Met Val Leu Pro Thr Phe
 1             5             10             15
15 Leu Arg Arg Arg Phe Asn Arg Leu Arg Met Gly Phe Phe Leu His Ser
    20             25             30
    Pro Phe Pro Ser Ser Glu Ile Tyr Arg Thr Leu Pro Val Arg Glu Glu
    35             40             45
20 Ile Leu Lys Ala Leu Leu Cys Ala Asp Ile Val Gly Phe His Thr Phe
    50             55             60
    Asp Tyr Ala Arg His Phe Leu Ser Cys Cys Ser Arg Met Leu Gly Leu
25 65             70             75             80
    Glu Tyr Gln Ser Lys Arg Gly Tyr Ile Gly Leu Glu Tyr Tyr Gly Arg
    85             90             95
30 Thr Val Gly Ile Lys Ile Met Pro Val Gly Ile His Met Gly His Ile
    100            105            110
    Glu Ser Met Lys Lys Leu Ala Ala Lys Glu Leu Met Leu Lys Ala Leu
    115            120            125
35 Lys Gln Gln Phe Glu Gly Lys Thr Val Leu Leu Gly Ala Asp Asp Leu
    130            135            140
    Asp Ile Phe Lys Gly Ile Asn Leu Lys Leu Leu Ala Met Glu Gln Met
40 145            150            155            160
    Leu Lys Gln His Pro Lys Trp Gln Gly Gln Ala Val Leu Val Gln Ile
    165            170            175
45 Ala Asn Pro Thr Arg Gly Lys Gly Val Asp Phe Glu Glu Ile Gln Ala
    180            185            190
    Glu Ile Ser Glu Ser Cys Lys Arg Ile Asn Lys Gln Phe Gly Lys Pro
    195            200            205
50 Gly Tyr Glu Pro Ile Val Tyr Ile Asp Arg Pro Val Ser Ser Ser Glu
    210            215            220
    Arg Met Ala Tyr Tyr Ser Ile Ala Glu Cys Val Val Val Thr Ala Val
55 225            230            235            240

```

30

Ser Asp Gly Met Asn Phe Val
245

(2) INFORMATION FOR SEQ ID NO: 12:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 395 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

15

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Nicotiana tabacum*

20

(B) STRAIN: Samsun NN

(F) TISSUE TYPE: Leaf

(ix) FEATURE:

(A) NAME/KEY: CDS

25

(B) LOCATION: 1..395

(D) OTHER INFORMATION: /partial

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

30	GCG AAA CCG GTG ATG AAA CTT TAC AGG GAA GCA ACT GAC GGA TCA TAT	48
	Ala Lys Pro Val Met Lys Leu Tyr Arg Glu Ala Thr Asp Gly Ser Tyr	
	1 5 10 15	
	ATA GAA ACT AAA GAG AGT GCA TTA GTG TGG CAC CAT CAT GAT GCA GAC	96
35	Ile Glu Thr Lys Glu Ser Ala Leu Val Trp His His His Asp Ala Asp	
	20 25 30	
	CCT GAC TTT GGC TCC TGC CAG GCA AAG GAA TTG TTG GAT CAT TTG GAA	144
40	Pro Asp Phe Gly Ser Cys Gln Ala Lys Glu Leu Leu Asp His Leu Glu	
	35 40 45	
	AGC GTA CTT GCA AAT GAA CCT GCA GTT GTT AAG AGG GGC CAA CAT ATT	192
	Ser Val Leu Ala Asn Glu Pro Ala Val Val Lys Arg Gly Gln His Ile	
	50 55 60	
45	GTT GAA GTC AAG CCA CAA GGT GTG ACC AAA GGA TTA GTT TCA GAG AAG	240
	Val Glu Val Lys Pro Gln Gly Val Thr Lys Gly Leu Val Ser Glu Lys	
	65 70 75 80	
50	GTT CTC TCG ATG ATG GTT GAT AGT GGG AAA CCG CCC GAT TTT GTT ATG	288
	Val Leu Ser Met Met Val Asp Ser Gly Lys Pro Pro Asp Phe Val Met	
	85 90 95	
	TGC ATT GGA GAT GAT AGG TCA GAC GAA GAC ATG TTT GAG AGC ATA TTA	336
55	Cys Ile Gly Asp Asp Arg Ser Asp Glu Asp Met Phe Glu Ser Ile Leu	
	100 105 110	

91

AGC ACC GTA TCC AGT CTG TCA GTC ACT GCT GCC CCT GAT GTC TTT GCC 384
 Ser Thr Val Ser Ser Leu Ser Val Thr Ala Ala Pro Asp Val Phe Ala
 115 120 125

5 TGC ACC GTC GG 395
 Cys Thr Val
 130

10 (2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

(B) TYPE: amino acid

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

20 Ala Lys Pro Val Met Lys Leu Tyr Arg Glu Ala Thr Asp Gly Ser Tyr
 1 5 10 15
 25 Ile Glu Thr Lys Glu Ser Ala Leu Val Trp His His His Asp Ala Asp
 20 25 30
 Pro Asp Phe Gly Ser Cys Gln Ala Lys Glu Leu Leu Asp His Leu Glu
 35 40 45
 30 Ser Val Leu Ala Asn Glu Pro Ala Val Val Lys Arg Gly Gln His Ile
 50 55 60
 Val Glu Val Lys Pro Gln Gly Val Thr Lys Gly Leu Val Ser Glu Lys
 65 70 75 80
 35 Val Leu Ser Met Met Val Asp Ser Gly Lys Pro Pro Asp Phe Val Met
 85 90 95
 40 Cys Ile Gly Asp Asp Arg Ser Asp Glu Asp Met Phe Glu Ser Ile Leu
 100 105 110
 Ser Thr Val Ser Ser Leu Ser Val Thr Ala Ala Pro Asp Val Phe Ala
 115 120 125
 45 Cys Thr Val
 130

(2) INFORMATION FOR SEQ ID NO: 14:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

92

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

5 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Nicotiana tabacum*

(B) STRAIN: Samsun NN

(F) TISSUE TYPE: Leaf

10 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..491

(D) OTHER INFORMATION: /partial

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGG CTG TCG GCG GAA CAC GGC TAT TTC TTG AGG ACG AGT CAA GAT GAA	48
Gly Leu Ser Ala Glu His Gly Tyr Phe Leu Arg Thr Ser Gln Asp Glu	
1 5 10 15	
20 GAA TGG GAA ACA TGT GTA CCA CCA GTG GAA TGT TGT TGG AAA GAA ATA	96
Glu Trp Glu Thr Cys Val Pro Pro Val Glu Cys Cys Trp Lys Glu Ile	
20 25 30	
25 GCT GAG CCT GTT ATG CAA CTT TAC ACT GAG ACT ACT GAT GGA TCA GTT	144
Ala Glu Pro Val Met Gln Leu Tyr Thr Glu Thr Thr Asp Gly Ser Val	
35 40 45	
30 ATT GAA GAT AAG GAA ACA TCA ATG GTC TGG TCT TAC GAG GAT GCG GAT	192
Ile Glu Asp Lys Glu Thr Ser Met Val Trp Ser Tyr Glu Asp Ala Asp	
50 55 60	
35 CCT GAT TTT GGA TCA TGT CAG GCT AAG GAA CTT CTT GAT CAC CTA GAA	240
Pro Asp Phe Gly Ser Cys Gln Ala Lys Glu Leu Leu Asp His Leu Glu	
65 70 75 80	
AGT GTA CTA GCT AAT GAA CCG GTC ACT GTC AGG AGT GGA CAG AAT ATA	288
Ser Val Leu Ala Asn Glu Pro Val Thr Val Arg Ser Gly Gln Asn Ile	
85 90 95	
40 GTG GAA GTT AAG CCC CAG GGT GTA TCC AAA GGG CTT GTT GCC AAG CGC	336
Val Glu Val Lys Pro Gln Gly Val Ser Lys Gly Leu Val Ala Lys Arg	
100 105 110	
45 CTG CTT TCC GCA ATG CAA GAG AAA GGA ATG TCA CCA GAT TTT GTC CTT	384
Leu Leu Ser Ala Met Gln Glu Lys Gly Met Ser Pro Asp Phe Val Leu	
115 120 125	
50 TGC ATA GGA GAT GAC CGA TCG GAT GAA GAC ATG TTC GAG GTG ATC ATG	432
Cys Ile Gly Asp Asp Arg Ser Asp Glu Asp Met Phe Glu Val Ile Met	
130 135 140	
AGC TCG ATG TCT GGC CCG TCC ATG GCT CCA ACA GCT GAA GTC TTT GCC	480
Ser Ser Met Ser Gly Pro Ser Met Ala Pro Thr Ala Glu Val Phe Ala	
145 150 155 160	

93

TGC ACC GTC GG
Cys Thr Val

491

5 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 163 amino acids

(B) TYPE: amino acid

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

15

Gly Leu Ser Ala Glu His Gly Tyr Phe Leu Arg Thr Ser Gln Asp Glu
1 5 10 15

20 Glu Trp Glu Thr Cys Val Pro Pro Val Glu Cys Cys Trp Lys Glu Ile
20 25 30

Ala Glu Pro Val Met Gln Leu Tyr Thr Glu Thr Thr Asp Gly Ser Val
35 40 45

25 Ile Glu Asp Lys Glu Thr Ser Met Val Trp Ser Tyr Glu Asp Ala Asp
50 55 60

30 Pro Asp Phe Gly Ser Cys Gln Ala Lys Glu Leu Leu Asp His Leu Glu
65 70 75 80

Ser Val Leu Ala Asn Glu Pro Val Thr Val Arg Ser Gly Gln Asn Ile
85 90 95

35 Val Glu Val Lys Pro Gln Gly Val Ser Lys Gly Leu Val Ala Lys Arg
100 105 110

Leu Leu Ser Ala Met Gln Glu Lys Gly Met Ser Pro Asp Phe Val Leu
115 120 125

40 Cys Ile Gly Asp Asp Arg Ser Asp Glu Asp Met Phe Glu Val Ile Met
130 135 140

45 Ser Ser Met Ser Gly Pro Ser Met Ala Pro Thr Ala Glu Val Phe Ala
145 150 155 160

Cys Thr Val

(2) INFORMATION FOR SEQ ID NO: 16:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 361 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

55 (D) TOPOLOGY: linear

94

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

5 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Nicotiana tabacum*

(B) STRAIN: Samsun NN

10 (F) TISSUE TYPE: Leaf

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TTTGATTATG ATGGGACGCT GCTGTCGGAG GAGAGTGTGG AAAAAACCCC GAGTGAAGAT 60

15 GACATCTCAA TTCTGAATGG TTTATGCAGT GATCCAAAGA ACGTAGTCTT TATCGTGAGT 120

GGCAGAGGAA AGGATACACT TAGCAAGTGG TTCTCTCCGT GTCCGAGACT CGGCCTATCA 180

20 GCAGAACATG GATATTTTAC TAGGTGGAGT AAGGATTCCG AGTGGGAATC TCGTCCATAG 240

CTGCAGACCT TGA CTGGAAA AAAATAGTGT TGCCTATTAT GGAGCGCTAC ACAGAGCACA 300

GATGGTTCGT CGATAGAACA GAAGGAAACC TCGTGTGGC TCATCAAATG CTGGCCCCGA 360

25 A 361

(2) INFORMATION FOR SEQ ID NO: 17:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Nicotiana tabacum*

(B) STRAIN: Samsun NN

45 (F) TISSUE TYPE: Leaf

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGAAACCCAC AGGATGTAAG CAAAGTTTTA GTTTTGTAGA TCTCTTGGCA TCAAGCAAAG 60

50 TAGAGGGAAG TCACCCGATT CGTGCTGTGC GTAGGGATGA CAGATCGGAC GACTTAGA 118

95

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 417 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 15 (A) ORGANISM: *Nicotiana tabacum*
(B) STRAIN: Samsun NN
(F) TISSUE TYPE: Leaf

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

20 TTGTGGCCGA TGTTCCACTA CATGTTGCCG TTCTCACCTG ACCATGGAGG CCGCTTTGAT 60
CGCTCTATGT GGGAAGCATA TGTTTCTGCC AACAAAGTTGT TTTCACAAAA AGTAGTTGAG 120
25 GTTCTTAATC CTGAGGATGA CTTTGTCTGG ATTCATGATT ATCATTTGAT GGTGTTGCCA 180
ACGTTCTTGA GGAGGCGGTT CAATCGTTTG AGAATGGGGT TTTTCCTTCA CAGTCCATTC 240
30 CTTCATCTGA GATTACAGG ACACTTCCTG TTAGAGAGGA AATACTCAAG GCTTGTCTCT 300
GTGCTGACAT TGTGGATT CACACTTTTG ACTACGCGAG ACACTTCCTC TCTTGTGCA 360
GTCGATTTTG GGTAGAGTAC AGTCTAAAAA AAGTTATATT GGGTTAAAT ACTATGG 417
35

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 411 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

45

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 50 (A) ORGANISM: *Nicotiana tabacum*
(B) STRAIN: Samsun NN
(F) TISSUE TYPE: Leaf

96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGGTCATATT GATCCATGAA GAAATTGCAG CGAAAGAGTG ATGCTTTAAT GCGTAAAGCA 60
 5 GCAATTTGAA GGGAAAACTG TGTGTGTTAGG TGCCGATGAC CTGGATATTT TCAAAGGTAT 120
 GAACTTAAAG CTTCTAGCTA TGGAACAGAT GCTCAAACAT CACCCCAAGT GGCAAGGGCA 180
 GGCTGTGTTG GTCCAAGATT GCAAATCCTA CGAGGGGTAA AGGAGTAGAT TTTGACGAAA 240
 10 TACGGCTGAG ACATCGGAAA GCTGTAAGAG AATCAATAAG CAATTCGGCA AGCCTGGATA 300
 TGAGCCTATA GTTTATATTG ATAGGCCCGT GTCAAGCAGT GAACGCATGG CATATTACAG 360
 15 TATTGCAGGA TGTGTTGTGG TCACGCTGTG AGCGATGGCA TGAATCTGTT C 411

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
 20 (A) LENGTH: 405 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: NO
 (iii) ANTI-SENSE: NO
 30 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Nicotiana tabacum*
 (B) STRAIN: Samsun NN
 (F) TISSUE TYPE: Leaf
 35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGGGGTGGTT CCTGCATACG CCGTTTCCTT CTTCTGAGAT ATATAAACT TTGCCTATTC 60
 40 GCGAAAGATC TTACAGCTCT CTTGAATTCA ATTTGATTGG GTTCCACACT TTTGACTATG 120
 CAGGCACTTC CTCTCGTGTT GCAGTCGGAT GTTAGGTATT TCTTATGATC AAAAAGGGGT 180
 TACATAGGCC TCGATATTAT GGCAGGACTG TAATATAAAA ATTCTGCCAG CGGGTATTCA 240
 45 TATGGGGCAG CTTCAAGCAAG TCTTGAGTCT TCCTGAAACG GAGGCAAAAT CTCGGAACTC 300
 GTGCAGCATT TAATCATCAG GGGGAGGACA TTGTTGCTGG GATTGATGAC TGGACATATT 360
 50 TAAAGGCTCA TTTGAATTTA TTACCATGGA ACAACTCTAT TGCAC 405

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
5 (A) LENGTH: 427 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA
10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
15 (A) ORGANISM: *Nicotiana tabacum*
(B) STRAIN: Samsun NN
(F) TISSUE TYPE: Leaf

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
20

ATCATATGGG GCAGCTTCAG CAATCTTGAT CTCCTGAAA CGGAGGCAAA AGTCTTCGGA	60
ACTCGGCAGC AGTTTAATCA TCAGGGGAGG ACATGTGTC TGGGAGTTGA TGACATGGAC	120
25 ATATTTAAAG GCATCAGTTT GAAGTTATTA GCAATGGAAC AACTTCTATT GCAGCACCCG	180
GAGAAGCAGG GGAAGGTTGT TTTGGTGCAG ATAGCCAATC CTGCTAGAGG CAAAGGAAAA	240
30 GATGTCAAAG AAGTGCAGGA AGAACTCAT TGACGGTGAA GCGAATTAAT GAAGCATTTG	300
GAAGACCTGG GTACGAACCA GTTATCTTGA TTGATAAGCC ACTAAAGTTT TATGAAAGGA	360
TTGCTTATTA TGTGTGTCAG GAGTGTGCC TAGTCACTGC TGTCAGCGAT GGCATGAACC	420
35 TCGTCTC	427

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO
50

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Nicotiana tabacum*
(B) STRAIN: Samsun NN
55 (F) TISSUE TYPE: Leaf

90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GATGTGGATG CATGACTACC AATCCAAGAG GGGGTATATT GGTCTTGACT ATTATGGTAA 60
5 ACTGTGACCA TTAAATCCT TCCAGTTGGT ATTCACATGG GACAACTCCA AAATGTTATG 120
TCACTACAGA CACGGGAAAG AAAGCAAAGG AGTTGAAAGA AAAATATGAG GGGAAAATTG 180
10 TGATGTTAGG TATTGATGAT ATGGACATGT TTAAAGGAAT TGGTCTAAAG TTTCTGGCAA 240
TGGGGAGGCT TCTAGATGAA AACCCCTGTCT TGAGGGGTAA AGTGGTATTG GTTCAATCAC 300
CAGGCCTGGA AATTA 315

15 (2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 352 base pairs
(B) TYPE: nucleic acid
20. (C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

25 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Nicotiana tabacum
(B) STRAIN: Samsun NN
30 (F) TISSUE TYPE: Leaf

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

35 AGAAGTAAAG GGAGTGAGTC CCCGAGGTTT AAAAAGAGGT CAACAGAATT GCAGTGAAAT 60
TAATAAAAAA TATGGCAAAC CGGGGTACAA GCCGATTGTT TGTATCAATG GTCCAGTTTC 120
40 GACACAAGAC AAGATTGCAC ATTATGCGGT CTTGAGTGTG TTGTTGTAA TGCTGTTAGA 180
GATGGGATGA ACTTGGTGCC TTATGAGTAT ACGGTCTTTA GGCAGGGCAG CGATAATTTG 240
GATAAGGCCT TGCAGCTAGA TGGTCCTACT GCTTCCAGAA AGAGTGTGAT TATTGTCTTG 300
45 AATTCGTTGG GTGCTCGCCA TCTTTAGTGG CGCCATCCGC GTCAACCCCT GG 352

(2) INFORMATION FOR SEQ ID NO: 24:

50 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2640 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

99

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

5 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Helianthus annuus*
 (F) TISSUE TYPE: Leaf

10 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 171..2508

15 (ix) FEATURE:
 (A) NAME/KEY: unsure
 (B) LOCATION: replace(2141..2151, "ccatnnntta")

20 (ix) FEATURE:
 (A) NAME/KEY: unsure
 (B) LOCATION: replace(2237..2243, "actnaaa")

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

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GGATCCTGCG GTTTCATCAC ACAATATGAT ACTGTTACAT CTGATGCCCC TTCAGATGTC      60
25 CCAAATAGGT TGATTGTCGT ATCGAATCAG TTACCCATAA TCGCTAGGCT AAGACTAACG      120
   ACAATGGAGG GTCCTTTTGG GATTTCACTT GGGACGAGAG TTCGATTTAC ATG CAC      176
                                   Met His
                                   1
30 ATC AAA GAT GCA TTA CCC GCA GCC GTT GAG GTT TTC TAT GTT GGC GCA      224
   Ile Lys Asp Ala Leu Pro Ala Ala Val Glu Val Phe Tyr Val Gly Ala
                                   5          10          15
35 CTA AGG GCT GAC GTT GGC CCT ACC GAA CAA GAT GAC GTG TCA AAG ACA      272
   Leu Arg Ala Asp Val Gly Pro Thr Glu Gln Asp Asp Val Ser Lys Thr
                                   20          25          30
40 TTG CTC GAT AGG TTT AAT TGC GTT GCG GTT TTT GTC CCT ACT TCA AAA      320
   Leu Leu Asp Arg Phe Asn Cys Val Ala Val Phe Val Pro Thr Ser Lys
   35          40          45          50
45 TGG GAC CAA TAT TAT CAC TGC TTT TGT AAG CAG TAT TTG TGG CCG ATA      368
   Trp Asp Gln Tyr Tyr His Cys Phe Cys Lys Gln Tyr Leu Trp Pro Ile
                                   55          60          65
   TTT CAT TAC AAG GTT CCC GCT TCT GAC GTC AAG AGT GTC CCG AAT AGT      416
   Phe His Tyr Lys Val Pro Ala Ser Asp Val Lys Ser Val Pro Asn Ser
                                   70          75          80
50 CGG GAT TCA TGG AAC GCT TAT GTT CAC GTG AAC AAA GAG TTT TCC CAG      464
   Arg Asp Ser Trp Asn Ala Tyr Val His Val Asn Lys Glu Phe Ser Gln
   85          90          95

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55

100

	AAG GTG ATG GAG GCA GTA ACC AAT GCT AGC AAT TAT GTA TGG ATA CAT	512
	Lys Val Met Glu Ala Val Thr Asn Ala Ser Asn Tyr Val Trp Ile His	
	100 105 110	
5	GAC TAC CAT TTA ATG ACG CTA CCG ACT TTC TTG AGG CGG GAT TTT TGT	560
	Asp Tyr His Leu Met Thr Leu Pro Thr Phe Leu Arg Arg Asp Phe Cys	
	115 120 125 130	
10	CGT TTT AAA ATC GGT TTT TTT CTG CAT AGC CCG TTT CCT TCC TCG GAG	608
	Arg Phe Lys Ile Gly Phe Phe Leu His Ser Pro Phe Pro Ser Ser Glu	
	135 140 145	
15	GTT TAC AAG ACC CTA CCA ATG AGA AAC GAG CTC TTG AAG GGT CTG TTA	656
	Val Tyr Lys Thr Leu Pro Met Arg Asn Glu Leu Leu Lys Gly Leu Leu	
	150 155 160	
20	AAT GCT GAT CTT ATC GGG TTC CAT ACA TAC GAT TAT GCC CGT CAT TTT	704
	Asn Ala Asp Leu Ile Gly Phe His Thr Tyr Asp Tyr Ala Arg His Phe	
	165 170 175	
25	CTA ACG TGT TGT AGT CGA ATG TTT GGT TTG GAT CAT CAG TTG AAA AGG	752
	Leu Thr Cys Cys Ser Arg Met Phe Gly Leu Asp His Gln Leu Lys Arg	
	180 185 190	
30	GGG TAC ATT TTC TTG GAA TAT AAT GGA AGG AGC ATT GAG ATC AAG ATA	800
	Gly Tyr Ile Phe Leu Glu Tyr Asn Gly Arg Ser Ile Glu Ile Lys Ile	
	195 200 205 210	
35	AAG GCG AGC GGG ATT CAT GTT GGT CGA ATG GAG TCG TAC TTG AGT CAG	848
	Lys Ala Ser Gly Ile His Val Gly Arg Met Glu Ser Tyr Leu Ser Gln	
	215 220 225	
40	CCC GAT ACA AGA TTA CAA GTT CAA GAA CTA AAA AAA CGT TTC GAA GGG	896
	Pro Asp Thr Arg Leu Gln Val Gln Glu Leu Lys Lys Arg Phe Glu Gly	
	230 235 240	
45	AAA ATC GTG CTA CTT GGA GTT GAT GAT TTG GAT ATA TTC AAA GGT GTG	944
	Lys Ile Val Leu Leu Gly Val Asp Asp Leu Asp Ile Phe Lys Gly Val	
	245 250 255	
50	AAC TTC AAG GTT TTA GCG TTG GAG AAG TTA CTT AAA TCA CAC CCG AGT	992
	Asn Phe Lys Val Leu Ala Leu Glu Lys Leu Leu Lys Ser His Pro Ser	
	260 265 270	
55	TGG CAA GGG CGT GTG GTT TTG GTG CAA ATC TTG AAT CCC GCT CGC GCG	1040
	Trp Gln Gly Arg Val Val Leu Val Gln Ile Leu Asn Pro Ala Arg Ala	
	275 280 285 290	
60	CGT TGC CAA GAC GTC GAT GAG ATC AAT GCC GAG ATA AGA ACA GTC TGT	1088
	Arg Cys Gln Asp Val Asp Glu Ile Asn Ala Glu Ile Arg Thr Val Cys	
	295 300 305	
65	GAA AGA ATC AAT AAC GAA CTG GGA AGC CCG GGA TAC CAG CCC GTT GTG	1136
	Glu Arg Ile Asn Asn Glu Leu Gly Ser Pro Gly Tyr Gln Pro Val Val	
	310 315 320	

	TTA ATT GAT GGG CCC GTT TCG TTA AGT GAA AAA GCT GCT TAT TAT GCT	1184
	Leu Ile Asp Gly Pro Val Ser Leu Ser Glu Lys Ala Ala Tyr Tyr Ala	
	325 330 335	
5	ATC GCC GAT ATG GCA ATT GTT ACA CCG TTA CGT GAC GGC ATG AAT CTT	1232
	Ile Ala Asp Met Ala Ile Val Thr Pro Leu Arg Asp Gly Met Asn Leu	
	340 345 350	
10	ATC CCG TAC GAG TAC GTC GTT TCC CGA CAA AGT GTT AAT GAC CCA AAT	1280
	Ile Pro Tyr Glu Tyr Val Val Ser Arg Gln Ser Val Asn Asp Pro Asn	
	355 360 365 370	
15	CCC AAT ACT CCA AAA AAG AGC ATG CTA GTG GTC TCC GAG TTC ATC GGG	1328
	Pro Asn Thr Pro Lys Lys Ser Met Leu Val Ser Glu Phe Ile Gly	
	375 380 385	
20	TGT TCA CTA TCT TTA ACC GGG GCC ATA CGG GTC AAC CCA TGG GAT GAG	1376
	Cys Ser Leu Ser Leu Thr Gly Ala Ile Arg Val Asn Pro Trp Asp Glu	
	390 395 400	
25	TTG GAG ACA GCA GAA GCA TTA TAC GAC GCA CTC ATG GCT CCT GAT GAC	1424
	Leu Glu Thr Ala Glu Ala Leu Tyr Asp Ala Leu Met Ala Pro Asp Asp	
	405 410 415	
30	CAT AAA GAA ACC GCC CAC ATG AAA CAG TAT CAA TAC ATT ATC TCC CAT	1472
	His Lys Glu Thr Ala His Met Lys Gln Tyr Gln Tyr Ile Ile Ser His	
	420 425 430	
35	GAT GTA GCT AAC TGG GCT CGT AGC TTC TTT CAA GAT TTA GAG CAA GCG	1520
	Asp Val Ala Asn Trp Ala Arg Ser Phe Phe Gln Asp Leu Glu Gln Ala	
	435 440 445 450	
40	TGC ATC GAT CAT TCT CGT AAA CGA TGC ATG AAT TTA GGA TTT GGG TTA	1568
	Cys Ile Asp His Ser Arg Lys Arg Cys Met Asn Leu Gly Phe Gly Leu	
	455 460 465	
45	GAT ACT AGA GTC GTT CTT TTT GAT GAG AAG TTT AGC AAG TTG GAT ATA	1616
	Asp Thr Arg Val Val Leu Phe Asp Glu Lys Phe Ser Lys Leu Asp Ile	
	470 475 480	
50	GAT GTC TTG GAG AAT GCT TAT TCC ATG GCT CAA AAT CGG GCC ATA CTT	1664
	Asp Val Leu Glu Asn Ala Tyr Ser Met Ala Gln Asn Arg Ala Ile Leu	
	485 490 495	
55	TTG GAC TAT GAC GGC ACT GTT ACT CCA TCT ATC AGT AAA TCT CCA ACT	1712
	Leu Asp Tyr Asp Gly Thr Val Thr Pro Ser Ile Ser Lys Ser Pro Thr	
	500 505 510	
60	GAA GCT GTT ATC TCC ATG ATC AAC AAA CTG TGC AAT GAT CCA AAG AAC	1760
	Glu Ala Val Ile Ser Met Ile Asn Lys Leu Cys Asn Asp Pro Lys Asn	
	515 520 525 530	
65	ATG GTG TTC ATC GTT AGT GGA CGC AGT AGA GAA AAT CTT GGC AGT TGG	1808
	Met Val Phe Ile Val Ser Gly Arg Ser Arg Glu Asn Leu Gly Ser Trp	
	535 540 545	

	TTC	GGC	GCG	TGT	GAG	AAA	CCC	GCC	ATT	GCA	GCT	GAG	CAC	GGA	TAC	TTT	1856
	Phe	Gly	Ala	Cys	Glu	Lys	Pro	Ala	Ile	Ala	Ala	Glu	His	Gly	Tyr	Phe	
				550					555					560			
5	ATA	AGG	TGG	GCG	GGT	GAT	CAA	GAA	TGG	GAA	ACG	TGC	GCA	CGT	GAG	AAT	1904
	Ile	Arg	Trp	Ala	Gly	Asp	Gln	Glu	Trp	Glu	Thr	Cys	Ala	Arg	Glu	Asn	
			565					570					575				
	AAT	GTC	GGG	TGG	ATG	GAA	ATG	GCT	GAG	CCG	GTT	ATG	AAT	CTT	TAT	ACA	1952
10	Asn	Val	Gly	Trp	Met	Glu	Met	Ala	Glu	Pro	Val	Met	Asn	Leu	Tyr	Thr	
		580						585					590				
	GAA	ACT	ACT	GAC	GGT	TCG	TAT	ATT	GAA	AAG	AAA	GAA	ACT	GCA	ATG	GTT	2000
15	Glu	Thr	Thr	Asp	Gly	Ser	Tyr	Ile	Glu	Lys	Lys	Glu	Thr	Ala	Met	Val	
	595					600					605				610		
	TGG	CAC	TAT	GAA	GAT	GCT	GAT	AAA	GAT	CTT	GGG	TTG	GAG	CAG	GCT	AAG	2048
	Trp	His	Tyr	Glu	Asp	Ala	Asp	Lys	Asp	Leu	Gly	Leu	Glu	Gln	Ala	Lys	
				615						620					625		
20	GAA	CTG	TTG	GAC	CAT	CTT	GAA	AAC	GTG	CTC	GCT	AAT	GAG	CCC	GTT	GAA	2096
	Glu	Leu	Leu	Asp	His	Leu	Glu	Asn	Val	Leu	Ala	Asn	Glu	Pro	Val	Glu	
				630					635					640			
25	GTG	AAA	CGA	GGT	CAA	TAC	ATT	GTA	GAA	GTT	AAA	CCA	CAG	GTA	CCC	CAT	2144
	Val	Lys	Arg	Gly	Gln	Tyr	Ile	Val	Glu	Val	Lys	Pro	Gln	Val	Pro	His	
			645					650					655				
	GGG	TTA	CCT	TCT	TGT	TAT	GAC	ATT	CAT	AGG	CAC	AGA	TTT	GTA	GAA	TCT	2192
30	Gly	Leu	Pro	Ser	Cys	Tyr	Asp	Ile	His	Arg	His	Arg	Phe	Val	Glu	Ser	
		660					665					670					
	TTT	AAC	TTA	AAT	TTC	TTT	AAA	TAT	GAA	TGC	AAT	TAT	AGG	GGG	TCA	CTG	2240
	Phe	Asn	Leu	Asn	Phe	Phe	Lys	Tyr	Glu	Cys	Asn	Tyr	Arg	Gly	Ser	Leu	
35	675					680					685				690		
	AAA	GGT	ATA	GTT	GCA	GAG	AAG	ATT	TTT	GCG	TTC	ATG	GCT	GAA	AAG	GGA	2288
	Lys	Gly	Ile	Val	Ala	Glu	Lys	Ile	Phe	Ala	Phe	Met	Ala	Glu	Lys	Gly	
					695					700				705			
40	AAA	CAG	GCT	GAT	TTC	GTG	TTG	AGC	GTT	GGA	GAT	GAT	AGA	AGT	GAT	GAA	2336
	Lys	Gln	Ala	Asp	Phe	Val	Leu	Ser	Val	Gly	Asp	Asp	Arg	Ser	Asp	Glu	
				710					715					720			
45	GAC	ATG	TTT	GTG	GCC	ATT	GGG	GAT	GGA	ATA	AAA	AAG	GGT	CGG	ATA	ACT	2384
	Asp	Met	Phe	Val	Ala	Ile	Gly	Asp	Gly	Ile	Lys	Lys	Gly	Arg	Ile	Thr	
			725					730					735				
	AAC	AAC	AAT	TCA	GTG	TTT	ACA	TGC	GTA	GTG	GGA	GAG	AAA	CCG	AGT	GCA	2432
50	Asn	Asn	Asn	Ser	Val	Phe	Thr	Cys	Val	Val	Gly	Glu	Lys	Pro	Ser	Ala	
		740						745				750					
	GCT	GAG	TAC	TTT	TTA	GAC	GAG	ACG	AAA	GAT	GTT	TCA	ATG	ATG	CTC	GAG	2480
	Ala	Glu	Tyr	Phe	Leu	Asp	Glu	Thr	Lys	Asp	Val	Ser	Met	Met	Leu	Glu	
55	755					760					765				770		

103

AAG CTC GGG TGT CTC AGC AAC CAA GGA T GATGATCCGG AAGCTTCTCG 2528
 Lys Leu Gly Cys Leu Ser Asn Gln Gly
 775

5 TGATCTTTAT GAGTTAAAAG TTTTCGACTT TTTCTTCATC AAGATTCATG GGAAAGTTGT 2588

TCAATATGAA CTTGTGTTTC TTGGTTCTGG ATTTTAGGGA GTCTATGGAT CC 2640

10 (2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 779 amino acids

(B) TYPE: amino acid

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

20 Met His Ile Lys Asp Ala Leu Pro Ala Ala Val Glu Val Phe Tyr Val
 1 5 10 15
 Gly Ala Leu Arg Ala Asp Val Gly Pro Thr Glu Gln Asp Asp Val Ser
 25 20 25 30
 Lys Thr Leu Leu Asp Arg Phe Asn Cys Val Ala Val Phe Val Pro Thr
 35 40 45
 30 Ser Lys Trp Asp Gln Tyr Tyr His Cys Phe Cys Lys Gln Tyr Leu Trp
 50 55 60
 Pro Ile Phe His Tyr Lys Val Pro Ala Ser Asp Val Lys Ser Val Pro
 65 70 75 80
 35 Asn Ser Arg Asp Ser Trp Asn Ala Tyr Val His Val Asn Lys Glu Phe
 85 90 95
 Ser Gln Lys Val Met Glu Ala Val Thr Asn Ala Ser Asn Tyr Val Trp
 40 100 105 110
 Ile His Asp Tyr His Leu Met Thr Leu Pro Thr Phe Leu Arg Arg Asp
 115 120 125
 45 Phe Cys Arg Phe Lys Ile Gly Phe Phe Leu His Ser Pro Phe Pro Ser
 130 135 140
 Ser Glu Val Tyr Lys Thr Leu Pro Met Arg Asn Glu Leu Leu Lys Gly
 145 150 155 160
 50 Leu Leu Asn Ala Asp Leu Ile Gly Phe His Thr Tyr Asp Tyr Ala Arg
 165 170 175
 His Phe Leu Thr Cys Cys Ser Arg Met Phe Gly Leu Asp His Gln Leu
 55 180 185 190

104

Lys Arg Gly Tyr Ile Phe Leu Glu Tyr Asn Gly Arg Ser Ile Glu Ile
 195 200 205
 Lys Ile Lys Ala Ser Gly Ile His Val Gly Arg Met Glu Ser Tyr Leu
 5 210 215 220
 Ser Gln Pro Asp Thr Arg Leu Gln Val Gln Glu Leu Lys Lys Arg Phe
 225 230 235 240
 10 Glu Gly Lys Ile Val Leu Leu Gly Val Asp Asp Leu Asp Ile Phe Lys
 245 250 255
 Gly Val Asn Phe Lys Val Leu Ala Leu Glu Lys Leu Leu Lys Ser His
 260 265 270
 15 Pro Ser Trp Gln Gly Arg Val Val Leu Val Gln Ile Leu Asn Pro Ala
 275 280 285
 Arg Ala Arg Cys Gln Asp Val Asp Glu Ile Asn Ala Glu Ile Arg Thr
 20 290 295 300
 Val Cys Glu Arg Ile Asn Asn Glu Leu Gly Ser Pro Gly Tyr Gln Pro
 305 310 315 320
 25 Val Val Leu Ile Asp Gly Pro Val Ser Leu Ser Glu Lys Ala Ala Tyr
 325 330 335
 Tyr Ala Ile Ala Asp Met Ala Ile Val Thr Pro Leu Arg Asp Gly Met
 340 345 350
 30 Asn Leu Ile Pro Tyr Glu Tyr Val Val Ser Arg Gln Ser Val Asn Asp
 355 360 365
 Pro Asn Pro Asn Thr Pro Lys Lys Ser Met Leu Val Val Ser Glu Phe
 35 370 375 380
 Ile Gly Cys Ser Leu Ser Leu Thr Gly Ala Ile Arg Val Asn Pro Trp
 385 390 395 400
 40 Asp Glu Leu Glu Thr Ala Glu Ala Leu Tyr Asp Ala Leu Met Ala Pro
 405 410 415
 Asp Asp His Lys Glu Thr Ala His Met Lys Gln Tyr Gln Tyr Ile Ile
 420 425 430
 45 Ser His Asp Val Ala Asn Trp Ala Arg Ser Phe Phe Gln Asp Leu Glu
 435 440 445
 Gln Ala Cys Ile Asp His Ser Arg Lys Arg Cys Met Asn Leu Gly Phe
 50 450 455 460
 Gly Leu Asp Thr Arg Val Val Leu Phe Asp Glu Lys Phe Ser Lys Leu
 465 470 475 480
 55 Asp Ile Asp Val Leu Glu Asn Ala Tyr Ser Met Ala Gln Asn Arg Ala
 485 490 495

Ile Leu Leu Asp Tyr Asp Gly Thr Val Thr Pro Ser Ile Ser Lys Ser
 500 505 510
 5 Pro Thr Glu Ala Val Ile Ser Met Ile Asn Lys Leu Cys Asn Asp Pro
 515 520 525
 Lys Asn Met Val Phe Ile Val Ser Gly Arg Ser Arg Glu Asn Leu Gly
 530 535 540
 10 Ser Trp Phe Gly Ala Cys Glu Lys Pro Ala Ile Ala Ala Glu His Gly
 545 550 555 560
 Tyr Phe Ile Arg Trp Ala Gly Asp Gln Glu Trp Glu Thr Cys Ala Arg
 565 570 575
 15 Glu Asn Asn Val Gly Trp Met Glu Met Ala Glu Pro Val Met Asn Leu
 580 585 590
 20 Tyr Thr Glu Thr Thr Asp Gly Ser Tyr Ile Glu Lys Lys Glu Thr Ala
 595 600 605
 Met Val Trp His Tyr Glu Asp Ala Asp Lys Asp Leu Gly Leu Glu Gln
 610 615 620
 25 Ala Lys Glu Leu Leu Asp His Leu Glu Asn Val Leu Ala Asn Glu Pro
 625 630 635 640
 Val Glu Val Lys Arg Gly Gln Tyr Ile Val Glu Val Lys Pro Gln Val
 645 650 655
 30 Pro His Gly Leu Pro Ser Cys Tyr Asp Ile His Arg His Arg Phe Val
 660 665 670
 35 Glu Ser Phe Asn Leu Asn Phe Phe Lys Tyr Glu Cys Asn Tyr Arg Gly
 675 680 685
 Ser Leu Lys Gly Ile Val Ala Glu Lys Ile Phe Ala Phe Met Ala Glu
 690 695 700
 40 Lys Gly Lys Gln Ala Asp Phe Val Leu Ser Val Gly Asp Asp Arg Ser
 705 710 715 720
 Asp Glu Asp Met Phe Val Ala Ile Gly Asp Gly Ile Lys Lys Gly Arg
 725 730 735
 45 Ile Thr Asn Asn Asn Ser Val Phe Thr Cys Val Val Gly Glu Lys Pro
 740 745 750
 50 Ser Ala Ala Glu Tyr Phe Leu Asp Glu Thr Lys Asp Val Ser Met Met
 755 760 765
 Leu Glu Lys Leu Gly Cys Leu Ser Asn Gln Gly
 770 775

106

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2130 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 15 (A) ORGANISM: *Helianthus annuus*

(ix) FEATURE:

- 20 (A) NAME/KEY: CDS
 (B) LOCATION: 171..2130
 (D) OTHER INFORMATION: /partial

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

25 GGATCCTGCG GTTTCATCAC ACAATATGAT ACTGTTACAT CTGATGCCCC TTCAGATGTC 60
 CCAAATAGGT TGATTGTCGT ATCGAATCAG TTACCCATAA TCGCTAGGCT AAGACTAACG 120
 ACAATGGAGG GTCCTTTTGG GATTTCACTT GGGACGAGAG TTCGATTTAC ATG CAC 176
 30 Met His
 1
 ATC AAA GAT GCA TTA CCC GCA GCC GTT GAG GTT TTC TAT GTT GGC GCA 224
 Ile Lys Asp Ala Leu Pro Ala Ala Val Glu Val Phe Tyr Val Gly Ala
 35 5 10 15
 CTA AGG GCT GAC GTT GGC CCT ACC GAA CAA GAT GAC GTG TCA AAG ACA 272
 Leu Arg Ala Asp Val Gly Pro Thr Glu Gln Asp Asp Val Ser Lys Thr
 20 25 30
 40 TTG CTC GAT AGG TTT AAT TGC GTT GCG GTT TTT GTC CCT ACT TCA AAA 320
 Leu Leu Asp Arg Phe Asn Cys Val Ala Val Phe Val Pro Thr Ser Lys
 35 40 45 50
 45 TGG GAC CAA TAT TAT CAC TGC TTT TGT AAG CAG TAT TTG TGG CCG ATA 368
 Trp Asp Gln Tyr Tyr His Cys Phe Cys Lys Gln Tyr Leu Trp Pro Ile
 55 60 65
 TTT CAT TAC AAG GTT CCC GCT TCT GAC GTC AAG AGT GTC CCG AAT AGT 416
 50 Phe His Tyr Lys Val Pro Ala Ser Asp Val Lys Ser Val Pro Asn Ser
 70 75 80
 CGG GAT TCA TGG AAC GCT TAT GTT CAC GTG AAC AAA GAG TTT TCC CAG 464
 Arg Asp Ser Trp Asn Ala Tyr Val His Val Asn Lys Glu Phe Ser Gln
 55 85 90 95

107

	AAG GTG ATG GAG GCA GTA ACC AAT GCT AGC AAT TAT GTA TGG ATA CAT	512
	Lys Val Met Glu Ala Val Thr Asn Ala Ser Asn Tyr Val Trp Ile His	
	100 105 110	
5	GAC TAC CAT TTA ATG ACG CTA CCG ACT TTC TTG AGG CGG GAT TTT TGT	560
	Asp Tyr His Leu Met Thr Leu Pro Thr Phe Leu Arg Arg Asp Phe Cys	
	115 120 125 130	
10	CGT TTT AAA ATC GGT TTT TTT CTG CAT AGC CCG TTT CCT TCC TCG GAG	608
	Arg Phe Lys Ile Gly Phe Phe Leu His Ser Pro Phe Pro Ser Ser Glu	
	135 140 145	
15	GTT TAC AAG ACC CTA CCA ATG AGA AAC GAG CTC TTG AAG GGT CTG TTA	656
	Val Tyr Lys Thr Leu Pro Met Arg Asn Glu Leu Leu Lys Gly Leu Leu	
	150 155 160	
20	AAT GCT GAT CTT ATC GGG TTC CAT ACA TAC GAT TAT GCC CGT CAT TTT	704
	Asn Ala Asp Leu Ile Gly Phe His Thr Tyr Asp Tyr Ala Arg His Phe	
	165 170 175	
25	CTA ACG TGT TGT AGT CGA ATG TTT GGT TTG GAT CAT CAG TTG AAA AGG	752
	Leu Thr Cys Cys Ser Arg Met Phe Gly Leu Asp His Gln Leu Lys Arg	
	180 185 190	
30	GGG TAC ATT TTC TTG GAA TAT AAT GGA AGG AGC ATT GAG ATC AAG ATA	800
	Gly Tyr Ile Phe Leu Glu Tyr Asn Gly Arg Ser Ile Glu Ile Lys Ile	
	195 200 205 210	
35	AAG GCG AGC GGG ATT CAT GTT GGT CGA ATG GAG TCG TAC TTG AGT CAG	848
	Lys Ala Ser Gly Ile His Val Gly Arg Met Glu Ser Tyr Leu Ser Gln	
	215 220 225	
40	CCC GAT ACA AGA TTA CAA GTT CAA GAA CTA AAA AAA CGT TTC GAA GGG	896
	Pro Asp Thr Arg Leu Gln Val Gln Glu Leu Lys Lys Arg Phe Glu Gly	
	230 235 240	
45	AAA ATC GTG CTA CTT GGA GTT GAT GAT TTG GAT ATA TTC AAA GGT GTG	944
	Lys Ile Val Leu Leu Gly Val Asp Asp Leu Asp Ile Phe Lys Gly Val	
	245 250 255	
50	AAC TTC AAG GTT TTA GCG TTG GAG AAG TTA CTT AAA TCA CAC CCG AGT	992
	Asn Phe Lys Val Leu Ala Leu Glu Lys Leu Leu Lys Ser His Pro Ser	
	260 265 270	
55	TGG CAA GGG CGT GTG GTT TTG GTG CAA ATC TTG AAT CCC GCT CGC GCG	1040
	Trp Gln Gly Arg Val Val Leu Val Gln Ile Leu Asn Pro Ala Arg Ala	
	275 280 285 290	
60	CGT TGC CAA GAC GTC GAT GAG ATC AAT GCC GAG ATA AGA ACA GTC TGT	1088
	Arg Cys Gln Asp Val Asp Glu Ile Asn Ala Glu Ile Arg Thr Val Cys	
	295 300 305	
65	GAA AGA ATC AAT AAC GAA CTG GGA AGC CCG GGA TAC CAG CCC GTT GTG	1136
	Glu Arg Ile Asn Asn Glu Leu Gly Ser Pro Gly Tyr Gln Pro Val Val	
	310 315 320	

	TTA	ATT	GAT	GGG	CCC	GTT	TCG	TTA	AGT	GAA	AAA	GCT	GCT	TAT	TAT	GCT	1184
	Leu	Ile	Asp	Gly	Pro	Val	Ser	Leu	Ser	Glu	Lys	Ala	Ala	Tyr	Tyr	Ala	
			325					330					335				
5	ATC	GCC	GAT	ATG	GCA	ATT	GTT	ACA	CCG	TTA	CGT	GAC	GGC	ATG	AAT	CTT	1232
	Ile	Ala	Asp	Met	Ala	Ile	Val	Thr	Pro	Leu	Arg	Asp	Gly	Met	Asn	Leu	
		340					345					350					
10	ATC	CCG	TAC	GAG	TAC	GTC	GTT	TCC	CGA	CAA	AGT	GTT	AAT	GAC	CCA	AAT	1280
	Ile	Pro	Tyr	Glu	Tyr	Val	Val	Ser	Arg	Gln	Ser	Val	Asn	Asp	Pro	Asn	
		355				360					365					370	
15	CCC	AAT	ACT	CCA	AAA	AAG	AGC	ATG	CTA	GTG	GTC	TCC	GAG	TTC	ATC	GGG	1328
	Pro	Asn	Thr	Pro	Lys	Lys	Ser	Met	Leu	Val	Val	Ser	Glu	Phe	Ile	Gly	
					375					380					385		
20	TGT	TCA	CTA	TCT	TTA	ACC	GGG	GCC	ATA	CGG	GTC	AAC	CCA	TGG	GAT	GAG	1376
	Cys	Ser	Leu	Ser	Leu	Thr	Gly	Ala	Ile	Arg	Val	Asn	Pro	Trp	Asp	Glu	
				390				395						400			
	TTG	GAG	ACA	GCA	GAA	GCA	TTA	TAC	GAC	GCA	CTC	ATG	GCT	CCT	GAT	GAC	1424
	Leu	Glu	Thr	Ala	Glu	Ala	Leu	Tyr	Asp	Ala	Leu	Met	Ala	Pro	Asp	Asp	
		405					410					415					
25	CAT	AAA	GAA	ACC	GCC	CAC	ATG	AAA	CAG	TAT	CAA	TAC	ATT	ATC	TCC	CAT	1472
	His	Lys	Glu	Thr	Ala	His	Met	Lys	Gln	Tyr	Gln	Tyr	Ile	Ile	Ser	His	
		420					425					430					
30	GAT	GTA	GCT	AAC	TGG	GCT	CGT	AGC	TTC	TTT	CAA	GAT	TTA	GAG	CAA	GCG	1520
	Asp	Val	Ala	Asn	Trp	Ala	Arg	Ser	Phe	Phe	Gln	Asp	Leu	Glu	Gln	Ala	
		435				440					445					450	
35	TGC	ATC	GAT	CAT	TCT	CGT	AAA	CGA	TGC	ATG	AAT	TTA	GGA	TTT	GGG	TTA	1568
	Cys	Ile	Asp	His	Ser	Arg	Lys	Arg	Cys	Met	Asn	Leu	Gly	Phe	Gly	Leu	
				455					460						465		
40	GAT	ACT	AGA	GTC	GTT	CTT	TTT	GAT	GAG	AAG	TTT	AGC	AAG	TTG	GAT	ATA	1616
	Asp	Thr	Arg	Val	Val	Leu	Phe	Asp	Glu	Lys	Phe	Ser	Lys	Leu	Asp	Ile	
				470				475						480			
	GAT	GTC	TTG	GAG	AAT	GCT	TAT	TCC	ATG	GCT	CAA	AAT	CGG	GCC	ATA	CTT	1664
	Asp	Val	Leu	Glu	Asn	Ala	Tyr	Ser	Met	Ala	Gln	Asn	Arg	Ala	Ile	Leu	
		485					490					495					
45	TTG	GAC	TAT	GAC	GGC	ACT	GTT	ACT	CCA	TCT	ATC	AGT	AAA	TCT	CCA	ACT	1712
	Leu	Asp	Tyr	Asp	Gly	Thr	Val	Thr	Pro	Ser	Ile	Ser	Lys	Ser	Pro	Thr	
		500					505					510					
50	GAA	GCT	GTT	ATC	TCC	ATG	ATC	AAC	AAA	CTG	TGC	AAT	GAT	CCA	AAG	AAC	1760
	Glu	Ala	Val	Ile	Ser	Met	Ile	Asn	Lys	Leu	Cys	Asn	Asp	Pro	Lys	Asn	
		515				520					525					530	
55	ATG	GTG	TTC	ATC	GTT	AGT	GGA	CGC	AGT	AGA	GAA	AAT	CTT	GGC	AGT	TGG	1808
	Met	Val	Phe	Ile	Val	Ser	Gly	Arg	Ser	Arg	Glu	Asn	Leu	Gly	Ser	Trp	
				535					540						545		

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TTC GGC GCG TGT GAG AAA CCC GCC ATT GCA GCT GAG CAC GGA TAC TTT 1856
 Phe Gly Ala Cys Glu Lys Pro Ala Ile Ala Ala Glu His Gly Tyr Phe
 550 555 560

5 ATA AGG TGG GCG GGT GAT CAA GAA TGG GAA ACG TGC GCA CGT GAG AAT 1904
 Ile Arg Trp Ala Gly Asp Gln Glu Trp Glu Thr Cys Ala Arg Glu Asn
 565 570 575

AAT GTC GGG TGG ATG GAA ATG GCT GAG CCG GTT ATG AAT CTT TAT ACA 1952
 10 Asn Val Gly Trp Met Glu Met Ala Glu Pro Val Met Asn Leu Tyr Thr
 580 585 590

GAA ACT ACT GAC GGT TCG TAT ATT GAA AAG AAA GAA ACT GCA ATG GTT 2000
 15 Glu Thr Thr Asp Gly Ser Tyr Ile Glu Lys Lys Glu Thr Ala Met Val
 595 600 605 610

TGG CAC TAT GAA GAT GCT GAT AAA GAT CTT GGG TTG GAG CAG GCT AAG 2048
 Trp His Tyr Glu Asp Ala Asp Lys Asp Leu Gly Leu Glu Gln Ala Lys
 615 620 625

20 GAA CTG TTG GAC CAT CTT GAA AAC GTG CTC GCT AAT GAG CCC GTT GAA 2096
 Glu Leu Leu Asp His Leu Glu Asn Val Leu Ala Asn Glu Pro Val Glu
 630 635 640

25 GTG AAA CGA GGT CAA TAC ATT GTA GAA GTT AAA C 2130
 Val Lys Arg Gly Gln Tyr Ile Val Glu Val Lys
 645 650

(2) INFORMATION FOR SEQ ID NO: 27:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 653 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

40 Met His Ile Lys Asp Ala Leu Pro Ala Ala Val Glu Val Phe Tyr Val
 1 5 10 15

Gly Ala Leu Arg Ala Asp Val Gly Pro Thr Glu Gln Asp Asp Val Ser
 20 25 30

45 Lys Thr Leu Leu Asp Arg Phe Asn Cys Val Ala Val Phe Val Pro Thr
 35 40 45

Ser Lys Trp Asp Gln Tyr Tyr His Cys Phe Cys Lys Gln Tyr Leu Trp
 50 50 55 60

Pro Ile Phe His Tyr Lys Val Pro Ala Ser Asp Val Lys Ser Val Pro
 65 70 75 80

55 Asn Ser Arg Asp Ser Trp Asn Ala Tyr Val His Val Asn Lys Glu Phe
 85 90 95

//O

	Ser	Gln	Lys	Val	Met	Glu	Ala	Val	Thr	Asn	Ala	Ser	Asn	Tyr	Val	Trp	100	105	110
5	Ile	His	Asp	Tyr	His	Leu	Met	Thr	Leu	Pro	Thr	Phe	Leu	Arg	Arg	Asp	115	120	125
	Phe	Cys	Arg	Phe	Lys	Ile	Gly	Phe	Phe	Leu	His	Ser	Pro	Phe	Pro	Ser	130	135	140
10	Ser	Glu	Val	Tyr	Lys	Thr	Leu	Pro	Met	Arg	Asn	Glu	Leu	Leu	Lys	Gly	145	150	155
	Leu	Leu	Asn	Ala	Asp	Leu	Ile	Gly	Phe	His	Thr	Tyr	Asp	Tyr	Ala	Arg	165	170	175
15	His	Phe	Leu	Thr	Cys	Cys	Ser	Arg	Met	Phe	Gly	Leu	Asp	His	Gln	Leu	180	185	190
	Lys	Arg	Gly	Tyr	Ile	Phe	Leu	Glu	Tyr	Asn	Gly	Arg	Ser	Ile	Glu	Ile	195	200	205
20	Lys	Ile	Lys	Ala	Ser	Gly	Ile	His	Val	Gly	Arg	Met	Glu	Ser	Tyr	Leu	210	215	220
	Ser	Gln	Pro	Asp	Thr	Arg	Leu	Gln	Val	Gln	Glu	Leu	Lys	Lys	Arg	Phe	225	230	235
	Glu	Gly	Lys	Ile	Val	Leu	Leu	Gly	Val	Asp	Asp	Leu	Asp	Ile	Phe	Lys	245	250	255
30	Gly	Val	Asn	Phe	Lys	Val	Leu	Ala	Leu	Glu	Lys	Leu	Leu	Lys	Ser	His	260	265	270
	Pro	Ser	Trp	Gln	Gly	Arg	Val	Val	Leu	Val	Gln	Ile	Leu	Asn	Pro	Ala	275	280	285
35	Arg	Ala	Arg	Cys	Gln	Asp	Val	Asp	Glu	Ile	Asn	Ala	Glu	Ile	Arg	Thr	290	295	300
	Val	Cys	Glu	Arg	Ile	Asn	Asn	Glu	Leu	Gly	Ser	Pro	Gly	Tyr	Gln	Pro	305	310	315
	Val	Val	Leu	Ile	Asp	Gly	Pro	Val	Ser	Leu	Ser	Glu	Lys	Ala	Ala	Tyr	325	330	335
45	Tyr	Ala	Ile	Ala	Asp	Met	Ala	Ile	Val	Thr	Pro	Leu	Arg	Asp	Gly	Met	340	345	350
	Asn	Leu	Ile	Pro	Tyr	Glu	Tyr	Val	Val	Ser	Arg	Gln	Ser	Val	Asn	Asp	355	360	365
50	Pro	Asn	Pro	Asn	Thr	Pro	Lys	Lys	Ser	Met	Leu	Val	Val	Ser	Glu	Phe	370	375	380
	Ile	Gly	Cys	Ser	Leu	Ser	Leu	Thr	Gly	Ala	Ile	Arg	Val	Asn	Pro	Trp	385	390	395
55																	400		

///

Asp Glu Leu Glu Thr Ala Glu Ala Leu Tyr Asp Ala Leu Met Ala Pro
 405 410 415
 5 Asp Asp His Lys Glu Thr Ala His Met Lys Gln Tyr Gln Tyr Ile Ile
 420 425 430
 Ser His Asp Val Ala Asn Trp Ala Arg Ser Phe Phe Gln Asp Leu Glu
 435 440 445
 10 Gln Ala Cys Ile Asp His Ser Arg Lys Arg Cys Met Asn Leu Gly Phe
 450 455 460
 Gly Leu Asp Thr Arg Val Val Leu Phe Asp Glu Lys Phe Ser Lys Leu
 465 470 475 480
 15 Asp Ile Asp Val Leu Glu Asn Ala Tyr Ser Met Ala Gln Asn Arg Ala
 485 490 495
 Ile Leu Leu Asp Tyr Asp Gly Thr Val Thr Pro Ser Ile Ser Lys Ser
 20 500 505 510
 Pro Thr Glu Ala Val Ile Ser Met Ile Asn Lys Leu Cys Asn Asp Pro
 515 520 525
 25 Lys Asn Met Val Phe Ile Val Ser Gly Arg Ser Arg Glu Asn Leu Gly
 530 535 540
 Ser Trp Phe Gly Ala Cys Glu Lys Pro Ala Ile Ala Ala Glu His Gly
 545 550 555 560
 30 Tyr Phe Ile Arg Trp Ala Gly Asp Gln Glu Trp Glu Thr Cys Ala Arg
 565 570 575
 Glu Asn Asn Val Gly Trp Met Glu Met Ala Glu Pro Val Met Asn Leu
 35 580 585 590
 Tyr Thr Glu Thr Thr Asp Gly Ser Tyr Ile Glu Lys Lys Glu Thr Ala
 595 600 605
 40 Met Val Trp His Tyr Glu Asp Ala Asp Lys Asp Leu Gly Leu Glu Gln
 610 615 620
 Ala Lys Glu Leu Leu Asp His Leu Glu Asn Val Leu Ala Asn Glu Pro
 625 630 635 640
 45 Val Glu Val Lys Arg Gly Gln Tyr Ile Val Glu Val Lys
 645 650

(2) INFORMATION FOR SEQ ID NO: 28:

50

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 390 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

55

112

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

5 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Helianthus annuus*

10 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 3..258

(D) OTHER INFORMATION: /partial

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TT GCA GAG AAG ATT TTT GCG TTC ATG GCT GAA AAG GGA AAA CAG GCT	47
Ala Glu Lys Ile Phe Ala Phe Met Ala Glu Lys Gly Lys Gln Ala	
1 5 10 15	
20 GAT TTC GTG TTG AGC GTT GGA GAT GAT AGA AGT GAT GAA GAC ATG TTT	95
Asp Phe Val Leu Ser Val Gly Asp Asp Arg Ser Asp Glu Asp Met Phe	
20 25 30	
25 GTG GCC ATT GGG GAT GGA ATA AAA AAG GGT CGG ATA ACT AAC AAC AAT	143
Val Ala Ile Gly Asp Gly Ile Lys Lys Gly Arg Ile Thr Asn Asn Asn	
35 40 45	
30 TCA GTG TTT ACA TGC GTA GTG GGA GAG AAA CCG AGT GCA GCT GAG TAC	191
Ser Val Phe Thr Cys Val Val Gly Glu Lys Pro Ser Ala Ala Glu Tyr	
50 55 60	
35 TTT TTA GAC GAG ACG AAA GAT GTT TCA ATG ATG CTC GAG AAG CTC GGG	239
Phe Leu Asp Glu Thr Lys Asp Val Ser Met Met Leu Glu Lys Leu Gly	
65 70 75	
TGT CTC AGC AAC CAA GGA T GATGATCCGG AAGCTTCTCG TGATCTTTAT	288
Cys Leu Ser Asn Gln Gly	
80 85	
40 GAGTTAAAAG TTTTCGACTT TTTCTTCATC AAGATTCATG GGAAAGTTGT TCAATATGAA	348
CTTGTGTTTC TTGGTTCTGG ATTTTAGGGA GTCTATGGAT CC	390

45 (2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 amino acids

(B) TYPE: amino acid

50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

55

1/3

Ala Glu Lys Ile Phe Ala Phe Met Ala Glu Lys Gly Lys Gln Ala Asp
 1 5 10 15
 Phe Val Leu Ser Val Gly Asp Asp Arg Ser Asp Glu Asp Met Phe Val
 5 20 25 30
 Ala Ile Gly Asp Gly Ile Lys Lys Gly Arg Ile Thr Asn Asn Asn Ser
 35 40 45
 10 Val Phe Thr Cys Val Val Gly Glu Lys Pro Ser Ala Ala Glu Tyr Phe
 50 55 60
 Leu Asp Glu Thr Lys Asp Val Ser Met Met Leu Glu Lys Leu Gly Cys
 65 70 75 80
 15 Leu Ser Asn Gln Gly
 85

(2) INFORMATION FOR SEQ ID NO: 30:

20

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

35 CCAIGGRTTI ACICKDATIG CICC

24

(2) INFORMATION FOR SEQ ID NO: 31:

- (i) SEQUENCE CHARACTERISTICS:
 40 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATHGTIGTIW SIAAYMRIYT ICC

23

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(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
5 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:
YTITGGCCIA TITTYCAYTA 20

(2) INFORMATION FOR SEQ ID NO: 33:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30 (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:
35 TGRTCIARIA RYTCYTTIGC 20

(2) INFORMATION FOR SEQ ID NO: 34:

40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:
TCRTCIGTRA ARTCRTCICC 20

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

TTYGAYTAYG AYGGIACIYT

20

(2) INFORMATION FOR SEQ ID NO: 36:

20

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

30

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

35 GGIYTIWBNG CIGARCAYGG

20

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

ATIGCIAARC CIGTIATGAA

20

(2) INFORMATION FOR SEQ ID NO: 38:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CCIACIGTRC AIGCRAAIAC

20

(2) INFORMATION FOR SEQ ID NO: 39:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2982 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Arabidopsis thaliana
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 64..2982

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

ATAAACTTCC TCGCGGCCGC CAGTGTGAGT AATTAGTTT TGGTCTGTT TTGGTGTGAG 60

CGT ATG CCT GGA AAT AAG TAC AAC TGC AGT TCT TCT CAT ATC CCA CTC 108

45 Met Pro Gly Asn Lys Tyr Asn Cys Ser Ser Ser His Ile Pro Leu

1 5 10 15

TCT CGA ACA GAA CGC CTC TTG AGA GAT AGA GAG CTT AGA GAG AAG AGG 156

50 Ser Arg Thr Glu Arg Leu Leu Arg Asp Arg Glu Leu Arg Glu Lys Arg

20 25 30

AAG AGC AAC CGA GCT CGT AAT CCT AAT GAC GTT GCT GGC AGT TCC GAG 204

Lys Ser Asn Arg Ala Arg Asn Pro Asn Asp Val Ala Gly Ser Ser Glu

35 40 45

55

	AAC TCT GAG AAT GAC TTG CGT TTA GAA GGT GAC AGT TCA AGG CAG TAT	252
	Asn Ser Glu Asn Asp Leu Arg Leu Glu Gly Asp Ser Ser Arg Gln Tyr	
	50 55 60	
5	GTT GAA CAG TAC TTG GAA GGG GCT GCT GCT GCA ATG GCG CAC GAT GAT	300
	Val Glu Gln Tyr Leu Glu Gly Ala Ala Ala Met Ala His Asp Asp	
	65 70 75	
10	GCG TGT GAG AGG CAA GAA GTT AGG CCT TAT AAT AGG CAA CGA CTA CTT	348
	Ala Cys Glu Arg Gln Glu Val Arg Pro Tyr Asn Arg Gln Arg Leu Leu	
	80 85 90 95	
15	GTA GTG GCT AAC AGG CTC CCA GTT TCT CCC GTG AGA AGA GGT GAA GAT	396
	Val Val Ala Asn Arg Leu Pro Val Ser Pro Val Arg Arg Gly Glu Asp	
	100 105 110	
20	TCA TGG TCT CTT GAG ATC AGT GCT GGT GGT CTA GTC AGT GCT CTC TTA	444
	Ser Trp Ser Leu Glu Ile Ser Ala Gly Gly Leu Val Ser Ala Leu Leu	
	115 120 125	
25	GGT GTA AAG GAA TTT GAG GCC AGA TGG ATA GGA TGG GCT GGA GTT AAT	492
	Gly Val Lys Glu Phe Glu Ala Arg Trp Ile Gly Trp Ala Gly Val Asn	
	130 135 140	
30	GTG CCT GAT GAG GTT GGA CAG AAG GCA CTT AGC AAA GCT TTG GCT GAG	540
	Val Pro Asp Glu Val Gly Gln Lys Ala Leu Ser Lys Ala Leu Ala Glu	
	145 150 155	
35	AAG AGG TGT ATT CCC GTG TTC CTT GAT GAA GAG ATT GTT CAT CAG TAC	588
	Lys Arg Cys Ile Pro Val Phe Leu Asp Glu Glu Ile Val His Gln Tyr	
	160 165 170 175	
40	TAT AAT GGT TAC TGC AAC AAT ATT CTG TGG CCT CTG TTT CAC TAC CTT	636
	Tyr Asn Gly Tyr Cys Asn Asn Ile Leu Trp Pro Leu Phe His Tyr Leu	
	180 185 190	
45	GGA CTT CCG CAA GAA GAT CGG CTT GCC ACA ACC AGA AGC TTT CAG TCC	684
	Gly Leu Pro Gln Glu Asp Arg Leu Ala Thr Thr Arg Ser Phe Gln Ser	
	195 200 205	
50	CAA TTT GCT GCA TAC AAG AAG GCA AAC CAA ATG TTC GCT GAT GTT GTA	732
	Gln Phe Ala Ala Tyr Lys Lys Ala Asn Gln Met Phe Ala Asp Val Val	
	210 215 220	
55	AAT GAG CAC TAT GAA GAG GGA GAT GTC GTC TGG TGC CAT GAC TAT CAT	780
	Asn Glu His Tyr Glu Glu Gly Asp Val Val Trp Cys His Asp Tyr His	
	225 230 235	
60	CTT ATG TTC CTT CCT AAA TGC CTT AAG GAG TAC AAC AGT AAG ATG AAA	828
	Leu Met Phe Leu Pro Lys Cys Leu Lys Glu Tyr Asn Ser Lys Met Lys	
	240 245 250 255	
65	GTT GGA TGG TTT CTC CAT ACA CCA TTC CCT TCG TCT GAG ATA CAC AGG	876
	Val Gly Trp Phe Leu His Thr Pro Phe Pro Ser Ser Glu Ile His Arg	
	260 265 270	

	ACA	CTT	CCA	TCA	CGA	TCA	GAG	CTC	CTT	CGG	TCA	GTT	CTT	GCT	GCT	GAT	924
	Thr	Leu	Pro	Ser	Arg	Ser	Glu	Leu	Leu	Arg	Ser	Val	Leu	Ala	Ala	Asp	
				275					280					285			
5	TTA	GTT	GGC	TTC	CAT	ACA	TAT	GAC	TAT	GCA	AGG	CAC	TTT	GTG	AGT	GCG	972
	Leu	Val	Gly	Phe	His	Thr	Tyr	Asp	Tyr	Ala	Arg	His	Phe	Val	Ser	Ala	
			290					295					300				
	TGC	ACT	CGT	ATT	CTT	GGA	CTT	GAA	GGA	ACA	CCT	GAG	GGA	GTT	GAG	GAT	1020
10	Cys	Thr	Arg	Ile	Leu	Gly	Leu	Glu	Gly	Thr	Pro	Glu	Gly	Val	Glu	Asp	
		305					310					315					
	CAA	GGC	AGG	CTC	ACT	CGT	GTA	GCT	GCT	TTT	CCA	ATT	GGC	ATA	GAT	TCT	1068
	Gln	Gly	Arg	Leu	Thr	Arg	Val	Ala	Ala	Phe	Pro	Ile	Gly	Ile	Asp	Ser	
15	320					325					330					335	
	GAT	CGG	TTT	ATA	CGA	GCA	CTT	GAG	GTC	CCC	GAA	GTC	AAA	CAA	CAC	ATG	1116
	Asp	Arg	Phe	Ile	Arg	Ala	Leu	Glu	Val	Pro	Glu	Val	Lys	Gln	His	Met	
				340						345					350		
20	AAG	GAA	TTG	AAA	GAA	AGA	TTT	ACT	GAC	AGA	AAG	GTG	ATG	TTA	GGT	GTT	1164
	Lys	Glu	Leu	Lys	Glu	Arg	Phe	Thr	Asp	Arg	Lys	Val	Met	Leu	Gly	Val	
			355						360					365			
25	GAT	CGT	CTT	GAC	ATG	ATC	AAA	GGG	ATT	CCA	CAA	AAG	ATT	CTG	GCA	TTC	1212
	Asp	Arg	Leu	Asp	Met	Ile	Lys	Gly	Ile	Pro	Gln	Lys	Ile	Leu	Ala	Phe	
		370						375					380				
	GAA	AAA	TTT	CTC	GAG	GAA	AAT	GCA	AAC	TGG	CGT	GAT	AAA	GTG	GTC	TTA	1260
30	Glu	Lys	Phe	Leu	Glu	Glu	Asn	Ala	Asn	Trp	Arg	Asp	Lys	Val	Val	Leu	
		385					390					395					
	TTG	AAA	ATT	GCG	GTG	CCA	ACA	AGA	CCT	GAC	GTT	CCT	GAG	TAT	CAA	ACA	1308
	Leu	Lys	Ile	Ala	Val	Pro	Thr	Arg	Pro	Asp	Val	Pro	Glu	Tyr	Gln	Thr	
35	400					405					410					415	
	CTC	ACA	AGC	CAA	GTT	CAT	GAA	ATT	GTT	GGC	CGC	ATT	ATT	GGT	CGT	CTC	1356
	Leu	Thr	Ser	Gln	Val	His	Glu	Ile	Val	Gly	Arg	Ile	Ile	Gly	Arg	Leu	
				420						425					430		
40	GGG	ACA	CTG	ACT	GCA	GTT	CCA	ATA	CAT	CAT	CTG	GAT	CGG	TCT	CTG	GAC	1404
	Gly	Thr	Leu	Thr	Ala	Val	Pro	Ile	His	His	Leu	Asp	Arg	Ser	Leu	Asp	
				435					440					445			
45	TTT	CAT	GCT	TTA	TGT	GCA	CTT	TAT	GCC	GTC	ACA	GAT	GTT	GCG	CTT	GTA	1452
	Phe	His	Ala	Leu	Cys	Ala	Leu	Tyr	Ala	Val	Thr	Asp	Val	Ala	Leu	Val	
			450					455					460				
	ACA	TCT	TTG	AGA	GAT	GGG	ATG	AAT	CTT	GTC	AGT	TAT	GAG	TTT	GTT	GCT	1500
50	Thr	Ser	Leu	Arg	Asp	Gly	Met	Asn	Leu	Val	Ser	Tyr	Glu	Phe	Val	Ala	
		465					470					475					
	TGC	CAA	GAG	GCC	AAA	AAG	GGC	GTC	CTC	ATT	CTC	AGT	GAA	TTT	GCA	GGT	1548
	Cys	Gln	Glu	Ala	Lys	Lys	Gly	Val	Leu	Ile	Leu	Ser	Glu	Phe	Ala	Gly	
55	480					485					490					495	

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	GCT GCA CAG TCT CTG GGT GCT GGA GCT ATT CTT GTG AAT CCT TGG AAC	1596
	Ala Ala Gln Ser Leu Gly Ala Gly Ala Ile Leu Val Asn Pro Trp Asn	
	500 505 510	
5	ATC ACA GAA GTT GCT GCC TCC ATT GGA CAA GCC CTA AAC ATG ACA GCT	1644
	Ile Thr Glu Val Ala Ala Ser Ile Gly Gln Ala Leu Asn Met Thr Ala	
	515 520 525	
10	GAA GAA AGA GAG AAA AGA CAT CGC CAT AAT TTT CAT CAT GTC AAA ACT	1692
	Glu Glu Arg Glu Lys Arg His Arg His Asn Phe His His Val Lys Thr	
	530 535 540	
15	CAC ACT GCT CAA GAA TGG GCT GAA ACT TTT GTC AGT GAA CTA AAT GAC	1740
	His Thr Ala Gln Glu Trp Ala Glu Thr Phe Val Ser Glu Leu Asn Asp	
	545 550 555	
20	ACT GTA ATT GAG GCG CAA CTA CGA ATT AGT AAA GTC CCA CCA GAG CTT	1788
	Thr Val Ile Glu Ala Gln Leu Arg Ile Ser Lys Val Pro Pro Glu Leu	
	560 565 570 575	
25	CCA CAG CAT GAT GCA ATT CAA CGG TAT TCA AAG TCC AAC AAC AGG CTT	1836
	Pro Gln His Asp Ala Ile Gln Arg Tyr Ser Lys Ser Asn Asn Arg Leu	
	580 585 590	
30	CTA ATC CTG GGT TTC AAT GCA ACA TTG ACT GAA CCA GTG GAT AAT CAA	1884
	Leu Ile Leu Gly Phe Asn Ala Thr Leu Thr Glu Pro Val Asp Asn Gln	
	595 600 605	
35	GGG AGA AGA GGT GAT CAA ATA AAG GAG ATG GAT CTT AAT CTA CAC CCT	1932
	Gly Arg Arg Gly Asp Gln Ile Lys Glu Met Asp Leu Asn Leu His Pro	
	610 615 620	
40	GAG CTT AAA GGG CCC TTA AAG GCA TTA TGC AGT GAT CCA AGT ACA ACC	1980
	Glu Leu Lys Gly Pro Leu Lys Ala Leu Cys Ser Asp Pro Ser Thr Thr	
	625 630 635	
45	ATA GTT GTT CTG AGC GGA AGC AGC AGA AGT GTT TTG GAC AAA AAC TTT	2028
	Ile Val Val Leu Ser Gly Ser Ser Arg Ser Val Leu Asp Lys Asn Phe	
	640 645 650 655	
50	GGA GAG TAT GAC ATG TGG CTG GCA GCA GAA AAT GGG ATG TTC CTA AGG	2076
	Gly Glu Tyr Asp Met Trp Leu Ala Ala Glu Asn Gly Met Phe Leu Arg	
	660 665 670	
55	CTT ACG AAT GGA GAG TGG ATG ACT ACA ATG CCA GAA CAC TTG AAC ATG	2124
	Leu Thr Asn Gly Glu Trp Met Thr Thr Met Pro Glu His Leu Asn Met	
	675 680 685	
60	GAA TGG GTT GAT AGC GTA AAG CAT GTT TTC AAG TAC TTC ACT GAG AGA	2172
	Glu Trp Val Asp Ser Val Lys His Val Phe Lys Tyr Phe Thr Glu Arg	
	690 695 700	
65	ACT CCC AGG TCA CAC TTT GAA ACT CGC GAT ACT TCG CTT ATT TGG AAC	2220
	Thr Pro Arg Ser His Phe Glu Thr Arg Asp Thr Ser Leu Ile Trp Asn	
	705 710 715	

/20

	TAC AAA TAT GCA GAT ATC GAA TTC GGG AGA CTT CAA GCA AGA GAT TTG	2268
	Tyr Lys Tyr Ala Asp Ile Glu Phe Gly Arg Leu Gln Ala Arg Asp Leu	
	720 725 730 735	
5	TTA CAA CAC TTA TGG ACA GGT CCA ATC TCT AAT GCA TCA GTT GAT GTT	2316
	Leu Gln His Leu Trp Thr Gly Pro Ile Ser Asn Ala Ser Val Asp Val	
	740 745 750	
10	GTC CAA GGA AGC CGC TCT GTG GAA GTC CGT GCA GTT GGT GTC ACA AAG	2364
	Val Gln Gly Ser Arg Ser Val Glu Val Arg Ala Val Gly Val Thr Lys	
	755 760 765	
15	GGA GCT GCA ATT GAT CGT ATT CTA GGA GAG ATA GTG CAT AGC AAG TCG	2412
	Gly Ala Ala Ile Asp Arg Ile Leu Gly Glu Ile Val His Ser Lys Ser	
	770 775 780	
20	ATG ACT ACA CCA ATC GAT TAC GTC TTG TGC ATT GGT CAT TTC TTG GGG	2460
	Met Thr Thr Pro Ile Asp Tyr Val Leu Cys Ile Gly His Phe Leu Gly	
	785 790 795	
	AAG GAC GAA GAT GTT TAC ACT TTC TTC GAA CCA GAA CTT CCA TCC GAC	2508
	Lys Asp Glu Asp Val Tyr Thr Phe Phe Glu Pro Glu Leu Pro Ser Asp	
	800 805 810 815	
25	ATG CCA GCC ATT GCA CGA TCC AGA CCA TCA TCT GAC AGT GGA GCC AAG	2556
	Met Pro Ala Ile Ala Arg Ser Arg Pro Ser Ser Asp Ser Gly Ala Lys	
	820 825 830	
30	TCA TCA TCA GGA GAC CGA AGA CCA CCT TCA AAG TCG ACA CAT AAC AAC	2604
	Ser Ser Ser Gly Asp Arg Arg Pro Pro Ser Lys Ser Thr His Asn Asn	
	835 840 845	
35	AAC AAA AGT GGA TCA AAA TCC TCA TCA TCC TCT AAC TCT AAC AAC AAC	2652
	Asn Lys Ser Gly Ser Lys Ser Ser Ser Ser Asn Ser Asn Asn Asn	
	850 855 860	
40	AAC AAG TCC TCA CAG AGA TCT CTT CAG TCA GAG AGA AAA AGT GGA TCC	2700
	Asn Lys Ser Ser Gln Arg Ser Leu Gln Ser Glu Arg Lys Ser Gly Ser	
	865 870 875	
	AAC CAT AGC TTA GGA AAC TCA AGA CGT CCT TCA CCA GAG AAG ATC TCA	2748
	Asn His Ser Leu Gly Asn Ser Arg Arg Pro Ser Pro Glu Lys Ile Ser	
	880 885 890 895	
45	TGG AAT GTG CTT GAC CTC AAA GGA GAG AAC TAC TTC TCT TGC GCT GTG	2796
	Trp Asn Val Leu Asp Leu Lys Gly Glu Asn Tyr Phe Ser Cys Ala Val	
	900 905 910	
50	GGT CGT ACT CGC ACC AAT GCT AGA TAT CTC CTT GGC TCA CCT GAC GAC	2844
	Gly Arg Thr Arg Thr Asn Ala Arg Tyr Leu Leu Gly Ser Pro Asp Asp	
	915 920 925	
55	GTC GTT TGC TTC CTT GAG AAG CTC GCT GAC ACC ACT TCC TCA CCT TAA	2892
	Val Val Cys Phe Leu Glu Lys Leu Ala Asp Thr Thr Ser Ser Pro *	
	930 935 940	

TAT CCC GAG ACA GTG TCA AGT GAG TTC ATG TAA CCC AAT AAA AAC TAT 2940
 Tyr Pro Glu Thr Val Ser Ser Glu Phe Met * Pro Asn Lys Asn Tyr
 945 950 955

5 TGT TTT GTA ACA AAA AGC AGC CAT TAC CAG ACT CTT TAG TGG 2982
 Cys Phe Val Thr Lys Ser Ser His Tyr Gln Thr Leu * Trp
 960 965 970

10 (2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 973 amino acids

(B) TYPE: amino acid

15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

20 Met Pro Gly Asn Lys Tyr Asn Cys Ser Ser Ser His Ile Pro Leu Ser
 1 5 10 15
 25 Arg Thr Glu Arg Leu Leu Arg Asp Arg Glu Leu Arg Glu Lys Arg Lys
 20 25 30
 Ser Asn Arg Ala Arg Asn Pro Asn Asp Val Ala Gly Ser Ser Glu Asn
 35 40 45
 30 Ser Glu Asn Asp Leu Arg Leu Glu Gly Asp Ser Ser Arg Gln Tyr Val
 50 55 60
 Glu Gln Tyr Leu Glu Gly Ala Ala Ala Ala Met Ala His Asp Asp Ala
 65 70 75 80
 35 Cys Glu Arg Gln Glu Val Arg Pro Tyr Asn Arg Gln Arg Leu Leu Val
 85 90 95
 Val Ala Asn Arg Leu Pro Val Ser Pro Val Arg Arg Gly Glu Asp Ser
 40 100 105 110
 Trp Ser Leu Glu Ile Ser Ala Gly Gly Leu Val Ser Ala Leu Leu Gly
 115 120 125
 45 Val Lys Glu Phe Glu Ala Arg Trp Ile Gly Trp Ala Gly Val Asn Val
 130 135 140
 Pro Asp Glu Val Gly Gln Lys Ala Leu Ser Lys Ala Leu Ala Glu Lys
 145 150 155 160
 50 Arg Cys Ile Pro Val Phe Leu Asp Glu Glu Ile Val His Gln Tyr Tyr
 165 170 175
 Asn Gly Tyr Cys Asn Asn Ile Leu Trp Pro Leu Phe His Tyr Leu Gly
 55 180 185 190

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	Leu	Pro	Gln	Glu	Asp	Arg	Leu	Ala	Thr	Thr	Arg	Ser	Phe	Gln	Ser	Gln	
			195					200					205				
5	Phe	Ala	Ala	Tyr	Lys	Lys	Ala	Asn	Gln	Met	Phe	Ala	Asp	Val	Val	Asn	
		210					215				220						
	Glu	His	Tyr	Glu	Glu	Gly	Asp	Val	Val	Trp	Cys	His	Asp	Tyr	His	Leu	
	225					230					235					240	
10	Met	Phe	Leu	Pro	Lys	Cys	Leu	Lys	Glu	Tyr	Asn	Ser	Lys	Met	Lys	Val	
					245					250					255		
	Gly	Trp	Phe	Leu	His	Thr	Pro	Phe	Pro	Ser	Ser	Glu	Ile	His	Arg	Thr	
				260					265					270			
15	Leu	Pro	Ser	Arg	Ser	Glu	Leu	Leu	Arg	Ser	Val	Leu	Ala	Ala	Asp	Leu	
			275					280					285				
	Val	Gly	Phe	His	Thr	Tyr	Asp	Tyr	Ala	Arg	His	Phe	Val	Ser	Ala	Cys	
20		290					295					300					
	Thr	Arg	Ile	Leu	Gly	Leu	Glu	Gly	Thr	Pro	Glu	Gly	Val	Glu	Asp	Gln	
	305				310						315					320	
25	Gly	Arg	Leu	Thr	Arg	Val	Ala	Ala	Phe	Pro	Ile	Gly	Ile	Asp	Ser	Asp	
					325					330					335		
	Arg	Phe	Ile	Arg	Ala	Leu	Glu	Val	Pro	Glu	Val	Lys	Gln	His	Met	Lys	
				340					345					350			
30	Glu	Leu	Lys	Glu	Arg	Phe	Thr	Asp	Arg	Lys	Val	Met	Leu	Gly	Val	Asp	
			355					360					365				
	Arg	Leu	Asp	Met	Ile	Lys	Gly	Ile	Pro	Gln	Lys	Ile	Leu	Ala	Phe	Glu	
35		370					375					380					
	Lys	Phe	Leu	Glu	Glu	Asn	Ala	Asn	Trp	Arg	Asp	Lys	Val	Val	Leu	Leu	
	385					390					395					400	
40	Lys	Ile	Ala	Val	Pro	Thr	Arg	Pro	Asp	Val	Pro	Glu	Tyr	Gln	Thr	Leu	
					405					410					415		
	Thr	Ser	Gln	Val	His	Glu	Ile	Val	Gly	Arg	Ile	Ile	Gly	Arg	Leu	Gly	
				420					425					430			
45	Thr	Leu	Thr	Ala	Val	Pro	Ile	His	His	Leu	Asp	Arg	Ser	Leu	Asp	Phe	
			435					440					445				
	His	Ala	Leu	Cys	Ala	Leu	Tyr	Ala	Val	Thr	Asp	Val	Ala	Leu	Val	Thr	
50		450					455					460					
	Ser	Leu	Arg	Asp	Gly	Met	Asn	Leu	Val	Ser	Tyr	Glu	Phe	Val	Ala	Cys	
	465					470					475					480	
55	Gln	Glu	Ala	Lys	Lys	Gly	Val	Leu	Ile	Leu	Ser	Glu	Phe	Ala	Gly	Ala	
					485					490					495		

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Ala Gln Ser Leu Gly Ala Gly Ala Ile Leu Val Asn Pro Trp Asn Ile
 500 505 510
 5 Thr Glu Val Ala Ala Ser Ile Gly Gln Ala Leu Asn Met Thr Ala Glu
 515 520 525
 Glu Arg Glu Lys Arg His Arg His Asn Phe His His Val Lys Thr His
 530 535 540
 10 Thr Ala Gln Glu Trp Ala Glu Thr Phe Val Ser Glu Leu Asn Asp Thr
 545 550 555 560
 Val Ile Glu Ala Gln Leu Arg Ile Ser Lys Val Pro Pro Glu Leu Pro
 565 570 575
 15 Gln His Asp Ala Ile Gln Arg Tyr Ser Lys Ser Asn Asn Arg Leu Leu
 580 585 590
 Ile Leu Gly Phe Asn Ala Thr Leu Thr Glu Pro Val Asp Asn Gln Gly
 595 600 605
 Arg Arg Gly Asp Gln Ile Lys Glu Met Asp Leu Asn Leu His Pro Glu
 610 615 620
 25 Leu Lys Gly Pro Leu Lys Ala Leu Cys Ser Asp Pro Ser Thr Thr Ile
 625 630 635 640
 Val Val Leu Ser Gly Ser Ser Arg Ser Val Leu Asp Lys Asn Phe Gly
 645 650 655
 30 Glu Tyr Asp Met Trp Leu Ala Ala Glu Asn Gly Met Phe Leu Arg Leu
 660 665 670
 Thr Asn Gly Glu Trp Met Thr Thr Met Pro Glu His Leu Asn Met Glu
 675 680 685
 Trp Val Asp Ser Val Lys His Val Phe Lys Tyr Phe Thr Glu Arg Thr
 690 695 700
 40 Pro Arg Ser His Phe Glu Thr Arg Asp Thr Ser Leu Ile Trp Asn Tyr
 705 710 715 720
 Lys Tyr Ala Asp Ile Glu Phe Gly Arg Leu Gln Ala Arg Asp Leu Leu
 725 730 735
 45 Gln His Leu Trp Thr Gly Pro Ile Ser Asn Ala Ser Val Asp Val Val
 740 745 750
 Gln Gly Ser Arg Ser Val Glu Val Arg Ala Val Gly Val Thr Lys Gly
 755 760 765
 50 Ala Ala Ile Asp Arg Ile Leu Gly Glu Ile Val His Ser Lys Ser Met
 770 775 780
 55 Thr Thr Pro Ile Asp Tyr Val Leu Cys Ile Gly His Phe Leu Gly Lys
 785 790 795 800

/ 2 4

Asp Glu Asp Val Tyr Thr Phe Phe Glu Pro Glu Leu Pro Ser Asp Met
 805 810 815
 Pro Ala Ile Ala Arg Ser Arg Pro Ser Ser Asp Ser Gly Ala Lys Ser
 5 820 825 830
 Ser Ser Gly Asp Arg Arg Pro Pro Ser Lys Ser Thr His Asn Asn Asn
 835 840 845
 10 Lys Ser Gly Ser Lys Ser Ser Ser Ser Ser Asn Ser Asn Asn Asn
 850 855 860
 Lys Ser Ser Gln Arg Ser Leu Gln Ser Glu Arg Lys Ser Gly Ser Asn
 865 870 875 880
 15 His Ser Leu Gly Asn Ser Arg Arg Pro Ser Pro Glu Lys Ile Ser Trp
 885 890 895
 Asn Val Leu Asp Leu Lys Gly Glu Asn Tyr Phe Ser Cys Ala Val Gly
 20 900 905 910
 Arg Thr Arg Thr Asn Ala Arg Tyr Leu Leu Gly Ser Pro Asp Asp Val
 915 920 925
 25 Val Cys Phe Leu Glu Lys Leu Ala Asp Thr Thr Ser Ser Pro * Tyr
 930 935 940
 Pro Glu Thr Val Ser Ser Glu Phe Met * Pro Asn Lys Asn Tyr Cys
 945 950 955 960
 30 Phe Val Thr Lys Ser Ser His Tyr Gln Thr Leu * Trp
 965 970

(2) INFORMATION FOR SEQ ID NO: 41:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 300 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

45 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Oryza sativa*

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ATAAACTTCC TCGGACCAAA GAAGAGCATG TTGGTTGTGT CGGAGTTTAT TGGTTGCTCA 60
 55 CCTTCACTGA GTGGAGCCAT TCGTGTAAAC CCGTGAATA TCGAGGCAAC TGCAGAGGCA 120

CTGAATGAGG CCATCTCAAT GTCAGAGCGT AAAAGCAGCT GAGGCACGAA AAACATTACC 180
 GTTATGTCAG CACCCATGAT GTTGCATATT GGTCTAAGAG CTTTGTACAG GACCTGGAGA 240
 5 GGGCTTGCAA GGATCACTTT AGGAAACCAT GCTGGGGCAT TGGATTGGAT TTCGCTCAGG 300

(2) INFORMATION FOR SEQ ID NO: 42:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 627 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 15 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: NO
 20 (iii) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Selaginella lepidophylla*
 25 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 4..627
 (D) OTHER INFORMATION: /partial
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

ATT ATG TGG GTG CAT GAT TAC CAC CTC TGT CTG GTC CCT CAG ATG ATC	48
Met Trp Val His Asp Tyr His Leu Cys Leu Val Pro Gln Met Ile	
1 5 10 15	
CGC CAA AAG CTG CCA GAT GTG CAG ATT GGC TTC TTC CTC CAC ACC GCT	96
Arg Gln Lys Leu Pro Asp Val Gln Ile Gly Phe Phe Leu His Thr Ala	
20 25 30	
TTT CCC TCG TCA GAG GTC TTC CGC TGC TTG GCC GCA CGA AAG GAG CTG	144
Phe Pro Ser Ser Glu Val Phe Arg Cys Leu Ala Ala Arg Lys Glu Leu	
35 40 45	
CTG GAC GGC ATG CTT GGT GCC AAC TTG GTT GCT TTC CAG ACG CCA GAG	192
Leu Asp Gly Met Leu Gly Ala Asn Leu Val Ala Phe Gln Thr Pro Glu	
50 55 60	
TAT GCA CAC CAC TTC CTC CAG ACG TGC AGT CGC ATT TCT CTG CTG AAG	240
Tyr Ala His His Phe Leu Gln Thr Cys Ser Arg Ile Ser Leu Leu Lys	
65 70 75	
CAA CCG AGG AAG GCG TTC AGC TCG TTT CGT CAA TGT CTG GTC ATA ATG	288
Gln Pro Arg Lys Ala Phe Ser Ser Phe Arg Gln Cys Leu Val Ile Met	
80 85 90 95	

55

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CAA GAA GCG CTA CGA GGG TCA AGA AGG TCA TCG TTG CGC GTG ACA AGC 336
 Gln Glu Ala Leu Arg Gly Ser Arg Arg Ser Ser Leu Arg Val Thr Ser
 100 105 110

5 TGA CAA CAT CGC GTG TAC GCG AGA AGC TTC TGT CGT ACG AGC TGT TCT 384
 * Gln His Arg Val Tyr Ala Arg Ser Phe Cys Arg Thr Ser Cys Ser
 115 120 125

TGA ACA AGA ACC CAC AGT GGA GGG ACA AGG TCG TTC TCA TTC AGG TTG 432
 10 * Thr Arg Thr His Ser Gly Gly Thr Arg Ser Phe Ser Phe Arg Leu
 130 135 140

CGA CCT CCA CGA CTG AGG ATT CTG AGC TTG CTG CGA CCG TAT CCG AAA 480
 Arg Pro Pro Arg Leu Arg Ile Leu Ser Leu Leu Arg Pro Tyr Pro Lys
 15 145 150 155

TTG TTA CAC GTA TTG ACG CTG TGC ACT CGA CGC TCA CAC ACA CCC ACT 528
 Leu Leu His Val Leu Thr Leu Cys Thr Arg Arg Ser His Thr Pro Thr
 160 165 170 175

20 CGT CTT CCT CAG GCA AGA CAT TGC GTT CTC GCA GTA CCT CGC ACT TCT 576
 Arg Leu Pro Gln Ala Arg His Cys Val Leu Ala Val Pro Arg Thr Ser
 180 185 190

25 CTC GAT CGC CGA TGC TCT TGC AAT CAA CTG TTC GAT GGC ATG AAC CTC 624
 Leu Asp Arg Arg Cys Ser Cys Asn Gln Leu Phe Asp Gly Met Asn Leu
 195 200 205

GTC 627
 30 Val

(2) INFORMATION FOR SEQ ID NO: 43:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

Met Trp Val His Asp Tyr His Leu Cys Leu Val Pro Gln Met Ile Arg
 45 1 5 10 15

Gln Lys Leu Pro Asp Val Gln Ile Gly Phe Phe Leu His Thr Ala Phe
 20 25 30

50 Pro Ser Ser Glu Val Phe Arg Cys Leu Ala Ala Arg Lys Glu Leu Leu
 35 40 45

Asp Gly Met Leu Gly Ala Asn Leu Val Ala Phe Gln Thr Pro Glu Tyr
 50 55 60

55

127

Ala His His Phe Leu Gln Thr Cys Ser Arg Ile Ser Leu Leu Lys Gln
65 70 75 80

Pro Arg Lys Ala Phe Ser Ser Phe Arg Gln Cys Leu Val Ile Met Gln
5 85 90 95

Glu Ala Leu Arg Gly Ser Arg Arg Ser Ser Leu Arg Val Thr Ser *

100 105 110

Gln His Arg Val Tyr Ala Arg Ser Phe Cys Arg Thr Ser Cys Ser *

115 120 125

Thr Arg Thr His Ser Gly Gly Thr Arg Ser Phe Ser Phe Arg Leu Arg
130 135 140

15 Pro Pro Arg Leu Arg Ile Leu Ser Leu Leu Arg Pro Tyr Pro Lys Leu
145 150 155 160

Leu His Val Leu Thr Leu Cys Thr Arg Arg Ser His Thr Pro Thr Arg
20 165 170 175

Leu Pro Gln Ala Arg His Cys Val Leu Ala Val Pro Arg Thr Ser Leu
180 185 190

25 Asp Arg Arg Cys Ser Cys Asn Gln Leu Phe Asp Gly Met Asn Leu Val
195 200 205

(2) INFORMATION FOR SEQ ID NO: 44:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 645 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Selaginella lepidophylla*

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GGGTGGTTCT TGCACACGCC GTTTCCTCTG TCTGAGATTT ACAGAACGCT GCCGCTGCCG 60

50 GCCGAGCTGC TCCAAGGCGT CTTAGGCGCG GACTTAGTGG GGTTCCACAC ATACGACTAT 120

GCAAGGCACT TTGTTAGCGC GATGCACACG GATACTCGGG CTGGAAGGCA CTCCCAGGCT 180

55 GTCGAGGATC AAGGGAAGAT CACGCGAGTG GCTGCCTTCC CCGTGGATCG ATTCGGAGCG 240

ATTTATCGAC GCGTAGAGAC CGATGCGGTC AAGAAACACA TGCAAGAGCT GAGCCAGGTT 300

126

TTGCTGTCGT AAGGTTATGT TGGGGTGGAT AGGCTTGACA TGATTAAAGG AATTCCACAG 360
 AAGCTGCTAG CCTTTGAAAA ATTCCTCGAG GAGAACTCCG AGTGGCGTGA TAAGGTCGTC 420
 5 CTGGTGCAAA TCGCGGTGCC GACTAGAACG GACGTCCTCG AGTACCAAAA GCTTACGAGC 480
 CAGGTTACAG AGATTGTTGG TCGCATAAAT GGACGTTTCG GCTCCTTGAC GGCTGTTCTT 540
 ATCCATCACC TCGATCGGTC CATGAAATTT CCGGAGCTTT GTGCGTTATA TGCAATCACT 600
 10 GATGTCCTGC TCGTGACATC CCTGCGCGAC GGCATGAACT TCGTC 645

(2) INFORMATION FOR SEQ ID NO: 45:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 498 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 20 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: NO
 25 (iii) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Arabidopsis thaliana*
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GCCGTTGTGG ATTCATCGCC TCGCACAAGC ACTCTTGTCTG TGTCTGAGTT TATTGGATGC 60
 TCACCTTCTT TGAGTGGTGC CATTAGGGTG AATCCATGGG ATGTGGATGC TGTTGCTGAA 120
 35 GCGGTAAACT CGGCTCTTAA AATAGTGAGA CTGAGAAGCA ACTACGGCAT GAGAAACATT 180
 ATCATTATAT TAGCACTCAT GATGTTGGTT ATTGGGCAAA GAGCTTTATG CAGGATCTTG 240
 40 AGAGAGCGTG CCGAGATCAT TATAGTAAAC GTTGTGGGG GATTGGTTTT GGCTTGGGGT 300
 TCAGAGTTTT GTCACTCTCT CCAAGTTTTA GGAAGCTATC TGTGGACACA TTTGTTCCAG 360
 TTTATAGGAA AACCACAGAG AGGGCTAATA TTCTTTTATA ATGGTACTCT TTGTTCCGAA 420
 45 AGCTCATTGT TCAAGATCCA GCAACGGGTT CCTTGTCTTA AGCCCCTTAA GGCCCCATAA 480
 CCGGTGTTTT TTAGTGAG 498

50 (2) INFORMATION FOR SEQ ID NO: 46:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 463 base pairs
 (B) TYPE: nucleic acid
 55 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

129

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

5 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
GCCGTTGTGG ATTCATCGCC TCGCACAAGC ACTCTTGTCG TGTCTGAGTT TATTGGATGC   60
TCACCTTCCTT TGAGTGGTGC CATTGGGTGA ATCCATGGGA TGTGGATGCT GTTGCTGAAG  120
15 CCGTAAACTC GGCTCTTAAA ATGAGTGAGA CTGAGAAGCA ACTACGGCAT GAGAAACATT  180
ATCATTATAT TAGCACTCAT GATGTTGGTT ATTGGGCAAA GAGCTTTATG CAGGATCTTG   240
20 AGAGAGCGTG CCGAGATCAT TATAGTAAAC GTTGTGGGGG GATTGGTTTT GGGTTGGGGT  300
TCAGAGTTTT TGTCACCTCTC TCCAAGTTTA GGAAGCTATC TTGGGACAAT TGTTCAGTT   360
TTTAGGGAAA ACACAGGGAA GGTATTTCCT TTGATTATAA TGGACCTTGT CCAAGCCCCA  420
25 TTTTAAAGGC CCAGGAACCG GGTTTTTTTT TCTTAAAGCC CCT                       463
```

(2) INFORMATION FOR SEQ ID NO: 47:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 394 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
GGTATTGATG TAGAGGAAAT ACGTGGTGAA ATCGAAGAAA GCTGCAGGAG GATCAATGGA   60
GAGTTTGGGA AACCGGATAT CAACCTATCA TATATATTGA TACCCGGTTT CGATTAATGA  120
50 AATAAATGCT TATACCATAT TGCTGAGTGC GTGGTCGTTA CAGCTGTTAG AGATGGTATG  180
AACCTTACTC CCTACGAATA TATCGTTTGT AGACAAGGTT TACTTGGGTC TGAATCAGAC  240
55 TTTAGTGGCC CAAAGAAGAG CATGTTGGTT GCATCAAGTT TATTTGGATG TCCCCTTTTCG  300
```

130

CTTAGTGGGG CTATACGCGT AAACCCATGG AACCGTTGAA GCTACTTGAG GAGCCTTAAT 360

TAGGCCCTC AAATATGCTG GAACACTACG GATG 394

5 (2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 428 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

15 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 20 (A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

25 AAGTCCGTTG TGGATTACAG CCTCGCACAA GCACTCTTGT CGTGTCTAGT TTATTGGATG 60
CTCACCTTCT TTAGTGGTGC CATTAGGGTG AATCCATGGA TGTGGATGCT GTTGCTGAAG 120
CGGTAAACTC GGCTCTTAAA ATAGTGAGAC TGAGAAGCAA CTACGGCATG AGAAACATTA 180
30 TCATTATATT AGCACTCATG ATGTTGGTTA TTGGGCAAAG AGCTTTATGC AGGACTTAGA 240
GAGCGTGCCG AGATCATTAT AGTAAACGTT GTTGGGGGAT TGGTTTGGT TTGGGGTTCA 300
AGTTTGTGCA CTCTCTCAA GTTTTAGGAA GCTATCTTGT GGACACATTG TTCCAGTTTA 360
35 TAGAAACACA GGAAGGGGC TATATTCTTG TTAAATGGG ACCCCTTGTC CCTAAAAGTC 420
CCATTTGT 428

40 (2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 481 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

50 (iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- 55 (A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CAAACGAAGA GCTTCGTGGG AAAGTGGTTC TCGTGCAGAT TACTAATCCT GCTCGTAGTT 60
5 CAGGTAAGGA TGTTCAAGAT GTAGAGAAAC AGATAAATTT ATTGCTGATG AGATCAATTC 120
TAAATTTGGG AGACCTGGTG GTTATAAGCC TATTGTTTTG TAATGGACCT GTTAGTACTT 180
TGGATAAAGT TGCTTATTAC GCGATCTCGG AGTGTGTTGT CGTGAATCTG TGAGAGATGG 240
10 GATGAATTTG GTGCCTTATA AGTACACAGT GACTCGGCAA GGGAGCCCTG CTTTGGATGC 300
AGCTTTGGTT TTGGGGAGGA TGATGTTAGG AAGACTGTGA TTATGTGTTT TGAGGTTCAA 360
15 CCGGTTGTCC TCCATCTCTA GTGGTGGGAT CCCTTTTAAT CCGTGGACAT CGATCAGCAC 420
TTACGCCCATG AGCTTCAAAT CCGGTTTCCG CAAAGGAAA ATTGCCCCGA GCTTAAGGCC 480
A 481
20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 395 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

35 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Arabidopsis thaliana*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

40 AGACCTGGTG GTTATAAGCC TATTGTGTTT GTCAATGGAC CTGTTAGTAC TTTGGATAAA 60
TTGCTTATTA CGCGATCTCG GAGTGTGTTG TCGTGAATCT GTGAGAGATG GGATGAATTT 120
GGTGCCTTAT AAGTACACAG TGACTCGGCA AGGGAGCCCT GCTTTGGATG CAGCTTTAGG 180
45 TTTTGGGGAG GATGATGTTA GGAAGAGTGT GATTATTGTT TCTAGTTCAT CGGTTGTCTC 240
CATCTCTGAG TGGTGGGATC CGTTAATCCG TGAACATCG TGCAGTCACT AAACGCCATG 300
50 AGCCTGCAAT ACGATGTCGC AAAGGGAAAA TCTTTGCCAC CAGAAGCATC ATAAGTACAT 360
AAAGCCTCAC AATTGCCTAT TTGGGCCGGG GTTTT 395

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
5 (A) LENGTH: 431 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA
10 (iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
15 (A) ORGANISM: *Oryza sativa*

(ix) FEATURE:
20 (A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /standard_name= "GENBANK ID:
D22143"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:
25
GGGAATGGAG GGTCTCCGAG CTGCAGCAGC AATTTGAGGG GAAGACTGTG TTGCTCGGTG 60
TGGATGACAT GGATATCTTC AAGGGTATCA ACTTGAAGCT TCTTGCCTTC GAGAATATGT 120
30 TGAGGACACA TCCCAAGTGG CAGGGGCGGG CAGTGTGGT GCAAATGCT AATCCGGCCC 180
GTGGAAAGGG TAAGGATCTT GAAGCCATCC AGGCTGAGAT TCATGAGAGC TGCAAGAGGA 240
TTAATGGAGA GTTTGGCCAG TCAGGATACA GCCCTGTTGT CTTCAATTGAC CGTGATGTGT 300
35 CAAGTGTGGA GGAAGATTGC CTACTACACA ATAGCAGAAT GTGTGGTGCT GACTGCTGTT 360
AGGGATGGGA TTGACTTGAC ACCATATGGA TATATTGTCT GTAGGGCAGG GGTCTTACTC 420
40 ACATCAGAGG T 431

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
45 (A) LENGTH: 496 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA
50 (iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Oryza sativa*

(ix) FEATURE:

5 (A) NAME/KEY: misc_feature

(B) LOCATION: 1

(D) OTHER INFORMATION: /standard_name= "GENBANK ID: D40048"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

10 CTACCGTTCC CTCCCTGTTT GCGACGAGAT CCTCAAATCA CTGCTAAACT GCGATCTGAT 60
TGGGTTCCAC ACCTTTGATT ACGCGCGGCA TTTCCTGTCC TGCTGCAGCC GGATGCTGGG 120
15 GATCGAGTAC CAGTCGAAGA GGGGATATAT CGGTCTCGAT TACTTTGGCC GCACTGTTGG 180
GATAAAGATC ATGCCTGTTG GGATTAACAT GACGCAGCTG CAGACGCAGA TCCGGCTGCC 240
TGATCTTGAG TGGCGTGTCT CGAACTCCGG AAGCAGTTTG ATGGGAAGAC TGTCATGCTC 300
20 GGTGTGGATG ATATGGACAT ATTTAAGGGG ATTAATCTGA AAGTTCTTGC GTTTTGAGCA 360
GATGCTGAGG ACACACCCAA AATGGCAGCC AAGGCAGTTT TGGTGCAGAT TCAAACCAAG 420
25 GGTGGTTGTT GGGAGGACTT AGGTACAGCT AGATATGAGT TCAGGGGTAA TGACATTTCA 480
GGCGGTATTT CCTTGG 496

(2) INFORMATION FOR SEQ ID NO: 53:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40 (iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Oryza sativa*

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GGACCAAAGA AGAGCATGTT GGTGTGTCTG GAGTTTATTG GTTGCTCACC TTTACTGAGT 60
50 GGAGCCATTC GTGTTAACCC GTGGAATATC GAGGCAACTG CAGAGGCACT GAATGAGGCC 120
ATCTCAATGT CAGAGCGTAA AAGCAGCTGA GGCACGAAAA ACATTACCGT TATGTCAGCA 180
CCCATGATGT TGCATATTGG TCTAAGAGCT TTGTACAGGA CCTGGAGAGG GCTTGCAAGG 240
55 ATCACTTTAG GAAACCATGC TGGGGCATTG GATTGGATTT CGCTCAGG 288

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2207 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

10

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

15

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum
 (B) STRAIN: Kardal

(ix) FEATURE:

20

- (A) NAME/KEY: CDS
 (B) LOCATION: 161..1906

(ix) FEATURE:

25

- (A) NAME/KEY: misc_feature
 (B) LOCATION: 842..850
 (D) OTHER INFORMATION: /function= "putative
 glycosylationsite"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

30

CTTTTCTGAG TAATAACATA GGCATTGATT TTTTTCAT TAATAACACC TGCAAACATT 60

CCCATTGCCG GCATTCTCTG TTCTTACAAA AAAAAACATT TTTTGTTC CATAAATTAG 120

35 TTATGGCATC AGTATTGAAC CCTTTAACTT GTTATACAAT ATG GGT AAA GCT ATA 175
 Met Gly Lys Ala Ile
 1 5

ATT TTT ATG ATT TTT ACT ATG TCT ATG AAT ATG ATT AAA GCT GAA ACT 223
 40 Ile Phe Met Ile Phe Thr Met Ser Met Asn Met Ile Lys Ala Glu Thr
 10 15 20

TGC AAA TCC ATT GAT AAG GGT CCT GTA ATC CCA ACA ACC CCT TTA GTG 271
 45 Cys Lys Ser Ile Asp Lys Gly Pro Val Ile Pro Thr Thr Pro Leu Val
 25 30 35

ATT TTT CTT GAA AAA GTT CAA GAA GCT GCT CTT CAA ACT TAT GGC CAT 319
 Ile Phe Leu Glu Lys Val Gln Glu Ala Ala Leu Gln Thr Tyr Gly His
 40 45 50

50

AAA GGG TTT GAT GCT AAA CTG TTT GTT GAT ATG TCA CTG AGA GAG AGT 367
 Lys Gly Phe Asp Ala Lys Leu Phe Val Asp Met Ser Leu Arg Glu Ser
 55 60 65

135

	CTT TCA GAA ACA GTT GAA GCT TTT AAT AAG CTT CCA AGA GTT GTG AAT	415
	Leu Ser Glu Thr Val Glu Ala Phe Asn Lys Leu Pro Arg Val Val Asn	
	70 75 80 85	
5	GGT TCA ATA TCA AAA AGT GAT TTG GAT GGT TTT ATA GGT AGT TAC TTG	463
	Gly Ser Ile Ser Lys Ser Asp Leu Asp Gly Phe Ile Gly Ser Tyr Leu	
	90 95 100	
	AGT AGT CCT GAT AAG GAT TTG GTT TAT GTT GAG CCT ATG GAT TTT GTG	511
10	Ser Ser Pro Asp Lys Asp Leu Val Tyr Val Glu Pro Met Asp Phe Val	
	105 110 115	
	GCT GAG CCT GAA GGC TTT TTG CCA AAG GTG AAG AAT TCT GAG GTG AGG	559
15	Ala Glu Pro Glu Gly Phe Leu Pro Lys Val Lys Asn Ser Glu Val Arg	
	120 125 130	
	GCA TGG GCA TTG GAG GTG CAT TCA CTT TGG AAG AAT TTA AGT AGG AAA	607
	Ala Trp Ala Leu Glu Val His Ser Leu Trp Lys Asn Leu Ser Arg Lys	
	135 140 145	
20	GTG GCT GAT CAT GTA TTG GAA AAA CCA GAG TTG TAT ACT TTG CTT CCA	655
	Val Ala Asp His Val Leu Glu Lys Pro Glu Leu Tyr Thr Leu Leu Pro	
	150 155 160 165	
25	TTG AAA AAT CCA GTT ATT ATA CCG GGA TCG CGT TTT AAG GAG GTT TAT	703
	Leu Lys Asn Pro Val Ile Ile Pro Gly Ser Arg Phe Lys Glu Val Tyr	
	170 175 180	
	TAT TGG GAT TCT TAT TGG GTA ATA AGG GGT TTG TTA GCA AGC AAA ATG	751
30	Tyr Trp Asp Ser Tyr Trp Val Ile Arg Gly Leu Leu Ala Ser Lys Met	
	185 190 195	
	TAT GAA ACT GCA AAA GGG ATT GTG ACT AAT CTG GTT TCT CTG ATA GAT	799
35	Tyr Glu Thr Ala Lys Gly Ile Val Thr Asn Leu Val Ser Leu Ile Asp	
	200 205 210	
	CAA TTT GGT TAT GTT CTT AAC GGT GCA AGA GCA TAC TAC AGT AAC AGA	847
	Gln Phe Gly Tyr Val Leu Asn Gly Ala Arg Ala Tyr Tyr Ser Asn Arg	
	215 220 225	
40	AGT CAG CCT CCT GTC CTG GCC ACG ATG ATT GTT GAC ATA TTC AAT CAG	895
	Ser Gln Pro Pro Val Leu Ala Thr Met Ile Val Asp Ile Phe Asn Gln	
	230 235 240 245	
45	ACA GGT GAT TTA AAT TTG GTT AGA AGA TCC CTT CCT GCT TTG CTC AAG	943
	Thr Gly Asp Leu Asn Leu Val Arg Arg Ser Leu Pro Ala Leu Leu Lys	
	250 255 260	
	GAG AAT CAT TTT TGG AAT TCA GGA ATA CAT AAG GTG ACT ATT CAA GAT	991
50	Glu Asn His Phe Trp Asn Ser Gly Ile His Lys Val Thr Ile Gln Asp	
	265 270 275	
	GCT CAG GGA TCA AAC CAC AGC TTG AGT CGG TAC TAT GCT ATG TGG AAT	1039
55	Ala Gln Gly Ser Asn His Ser Leu Ser Arg Tyr Tyr Ala Met Trp Asn	
	280 285 290	

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	AAG	CCC	CGT	CCA	GAA	TCG	TCA	ACT	ATA	GAC	AGT	GAA	ACA	GCT	TCC	GTA	1087
	Lys	Pro	Arg	Pro	Glu	Ser	Ser	Thr	Ile	Asp	Ser	Glu	Thr	Ala	Ser	Val	
	295						300					305					
5	CTC	CCA	AAT	ATA	TGT	GAA	AAA	AGA	GAA	TTA	TAC	CGT	GAA	CTG	GCA	TCA	1135
	Leu	Pro	Asn	Ile	Cys	Glu	Lys	Arg	Glu	Leu	Tyr	Arg	Glu	Leu	Ala	Ser	
	310					315					320					325	
	GCT	GCT	GAA	AGT	GGA	TGG	GAT	TTC	AGT	TCA	AGA	TGG	ATG	AGC	AAC	GGA	1183
10	Ala	Ala	Glu	Ser	Gly	Trp	Asp	Phe	Ser	Ser	Arg	Trp	Met	Ser	Asn	Gly	
					330					335					340		
	TCT	GAT	CTG	ACA	ACA	ACT	AGT	ACA	ACA	TCA	ATT	CTA	CCA	GTT	GAT	TTG	1231
15	Ser	Asp	Leu	Thr	Thr	Thr	Ser	Thr	Thr	Ser	Ile	Leu	Pro	Val	Asp	Leu	
				345					350					355			
	AAT	GCA	TTC	CTT	CTG	AAG	ATG	GAA	CTT	GAC	ATT	GCC	TTT	CTA	GCA	AAT	1279
	Asn	Ala	Phe	Leu	Leu	Lys	Met	Glu	Leu	Asp	Ile	Ala	Phe	Leu	Ala	Asn	
			360					365				370					
20	CTT	GTT	GGA	GAA	AGT	AGC	ACG	GCT	TCA	CAT	TTT	ACA	GAA	GCT	GCT	CAA	1327
	Leu	Val	Gly	Glu	Ser	Ser	Thr	Ala	Ser	His	Phe	Thr	Glu	Ala	Ala	Gln	
		375					380				385						
25	AAT	AGA	CAG	AAG	GCT	ATA	AAC	TGT	ATC	TTT	TGG	AAC	GCA	GAG	ATG	GGG	1375
	Asn	Arg	Gln	Lys	Ala	Ile	Asn	Cys	Ile	Phe	Trp	Asn	Ala	Glu	Met	Gly	
	390					395					400				405		
	CAA	TGG	CTT	GAT	TAC	TGG	CTT	ACC	AAC	AGC	GAC	ACA	TCT	GAG	GAT	ATT	1423
30	Gln	Trp	Leu	Asp	Trp	Leu	Thr	Asn	Ser	Ser	Asp	Thr	Ser	Glu	Asp	Ile	
				410					415					420			
	TAT	AAA	TGG	GAA	GAT	TTG	CAC	CAG	AAC	AAG	AAG	TCA	TTT	GCC	TCT	AAT	1471
35	Tyr	Lys	Trp	Glu	Asp	Leu	His	Gln	Asn	Lys	Lys	Ser	Phe	Ala	Ser	Asn	
			425					430					435				
	TTT	GTT	CCG	CTG	TGG	ACT	GAA	ATT	TCT	TGT	TCA	GAT	AAT	AAT	ATC	ACA	1519
	Phe	Val	Pro	Leu	Trp	Thr	Glu	Ile	Ser	Cys	Ser	Asp	Asn	Asn	Ile	Thr	
			440				445					450					
40	ACT	CAG	AAA	GTA	GTT	CAA	AGT	CTC	ATG	AGC	TCG	GGC	TTG	CTT	CAG	CCT	1567
	Thr	Gln	Lys	Val	Val	Gln	Ser	Leu	Met	Ser	Ser	Gly	Leu	Leu	Gln	Pro	
		455				460					465						
45	GCA	GGG	ATT	GCA	ATG	ACC	TTG	TCT	AAT	ACT	GGA	CAG	CAA	TGG	GAT	TTT	1615
	Ala	Gly	Ile	Ala	Met	Thr	Leu	Ser	Asn	Thr	Gly	Gln	Gln	Trp	Asp	Phe	
	470					475					480				485		
	CCG	AAT	GGT	TGG	CCC	CCC	CTT	CAA	CAC	ATA	ATC	ATT	GAA	GGT	CTC	TTA	1663
50	Pro	Asn	Gly	Trp	Pro	Pro	Leu	Gln	His	Ile	Ile	Ile	Glu	Gly	Leu	Leu	
				490					495				500				
	AGG	TCT	GGA	CTA	GAA	GAG	GCA	AGA	ACC	TTA	GCA	AAA	GAC	ATT	GCT	ATT	1711
55	Arg	Ser	Gly	Leu	Glu	Glu	Ala	Arg	Thr	Leu	Ala	Lys	Asp	Ile	Ala	Ile	
			505					510				515					

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CGC TGG TTA AGA ACT AAC TAT GTG ACT TAC AAG AAA ACC GGT GCT ATG 1759
 Arg Trp Leu Arg Thr Asn Tyr Val Thr Tyr Lys Lys Thr Gly Ala Met
 520 525 530

5 TAT GAA AAA TAT GAT GTC ACA AAA TGT GGA GCA TAT GGA GGT GGT GGT 1807
 Tyr Glu Lys Tyr Asp Val Thr Lys Cys Gly Ala Tyr Gly Gly Gly Gly
 535 540 545

10 GAA TAT ATG TCC CAA ACG GGT TTC GGA TGG TCA AAT GGC GTT GTA CTG 1855
 Glu Tyr Met Ser Gln Thr Gly Phe Gly Trp Ser Asn Gly Val Val Leu
 550 555 560 565

15 GCA CTT CTA GAG GAA TTT GGA TGG CCT GAA GAT TTG AAG ATT GAT TGC 1903
 Ala Leu Leu Glu Glu Phe Gly Trp Pro Glu Asp Leu Lys Ile Asp Cys
 570 575 580

TAATGAGCAA GTAGAAAAGC CAAATGAAAC ATCATTGAGT TTTATTTTCT TCTTTTGTTA 1963

20 AAATAAGCTG CAATGGTTTG CTGATAGTTT ATGTTTTGTA TTACTATTTT ATAAGGTTTT 2023

TGTACCATAT CAAGTGATAT TACCATGAAC TATGTCGTTT GGACTCTTCA AATCGGATTT 2083

TGCAAAAATA ATGCAGTTTT GGAGAATCCG ATAACATAGA CCATGTATGG ATCTAAATTG 2143

25 TAAACAGCTT ACTATATTAA GTAAAAGAAA GATGATTCCT CTGCTTTAAA AAAAAAAAAA 2203

AAAA 2207

30 (2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 581 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

40 Met Gly Lys Ala Ile Ile Phe Met Ile Phe Thr Met Ser Met Asn Met
 1 5 10 15

45 Ile Lys Ala Glu Thr Cys Lys Ser Ile Asp Lys Gly Pro Val Ile Pro
 20 25 30

Thr Thr Pro Leu Val Ile Phe Leu Glu Lys Val Gln Glu Ala Ala Leu
 35 40 45

50 Gln Thr Tyr Gly His Lys Gly Phe Asp Ala Lys Leu Phe Val Asp Met
 50 55 60

Ser Leu Arg Glu Ser Leu Ser Glu Thr Val Glu Ala Phe Asn Lys Leu
 65 70 75 80

55

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	Pro	Arg	Val	Val	Asn	Gly	Ser	Ile	Ser	Lys	Ser	Asp	Leu	Asp	Gly	Phe	
					85					90					95		
5	Ile	Gly	Ser	Tyr	Leu	Ser	Ser	Pro	Asp	Lys	Asp	Leu	Val	Tyr	Val	Glu	
				100					105					110			
	Pro	Met	Asp	Phe	Val	Ala	Glu	Pro	Glu	Gly	Phe	Leu	Pro	Lys	Val	Lys	
			115					120					125				
10	Asn	Ser	Glu	Val	Arg	Ala	Trp	Ala	Leu	Glu	Val	His	Ser	Leu	Trp	Lys	
			130				135					140					
	Asn	Leu	Ser	Arg	Lys	Val	Ala	Asp	His	Val	Leu	Glu	Lys	Pro	Glu	Leu	
	145				150					155						160	
15	Tyr	Thr	Leu	Leu	Pro	Leu	Lys	Asn	Pro	Val	Ile	Ile	Pro	Gly	Ser	Arg	
					165					170					175		
	Phe	Lys	Glu	Val	Tyr	Tyr	Trp	Asp	Ser	Tyr	Trp	Val	Ile	Arg	Gly	Leu	
20				180					185					190			
	Leu	Ala	Ser	Lys	Met	Tyr	Glu	Thr	Ala	Lys	Gly	Ile	Val	Thr	Asn	Leu	
			195					200					205				
25	Val	Ser	Leu	Ile	Asp	Gln	Phe	Gly	Tyr	Val	Leu	Asn	Gly	Ala	Arg	Ala	
			210				215					220					
	Tyr	Tyr	Ser	Asn	Arg	Ser	Gln	Pro	Pro	Val	Leu	Ala	Thr	Met	Ile	Val	
	225				230					235					240		
30	Asp	Ile	Phe	Asn	Gln	Thr	Gly	Asp	Leu	Asn	Leu	Val	Arg	Arg	Ser	Leu	
				245					250						255		
	Pro	Ala	Leu	Leu	Lys	Glu	Asn	His	Phe	Trp	Asn	Ser	Gly	Ile	His	Lys	
35				260					265					270			
	Val	Thr	Ile	Gln	Asp	Ala	Gln	Gly	Ser	Asn	His	Ser	Leu	Ser	Arg	Tyr	
			275					280					285				
40	Tyr	Ala	Met	Trp	Asn	Lys	Pro	Arg	Pro	Glu	Ser	Ser	Thr	Ile	Asp	Ser	
			290				295						300				
	Glu	Thr	Ala	Ser	Val	Leu	Pro	Asn	Ile	Cys	Glu	Lys	Arg	Glu	Leu	Tyr	
	305				310					315					320		
45	Arg	Glu	Leu	Ala	Ser	Ala	Ala	Glu	Ser	Gly	Trp	Asp	Phe	Ser	Ser	Arg	
				325						330					335		
	Trp	Met	Ser	Asn	Gly	Ser	Asp	Leu	Thr	Thr	Thr	Ser	Thr	Thr	Ser	Ile	
50				340					345						350		
	Leu	Pro	Val	Asp	Leu	Asn	Ala	Phe	Leu	Leu	Lys	Met	Glu	Leu	Asp	Ile	
			355					360					365				
55	Ala	Phe	Leu	Ala	Asn	Leu	Val	Gly	Glu	Ser	Ser	Thr	Ala	Ser	His	Phe	
			370				375					380					

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Thr Glu Ala Ala Gln Asn Arg Gln Lys Ala Ile Asn Cys Ile Phe Trp
 385 390 395 400
 Asn Ala Glu Met Gly Gln Trp Leu Asp Tyr Trp Leu Thr Asn Ser Asp
 5 405 410 415
 Thr Ser Glu Asp Ile Tyr Lys Trp Glu Asp Leu His Gln Asn Lys Lys
 420 425 430
 10 Ser Phe Ala Ser Asn Phe Val Pro Leu Trp Thr Glu Ile Ser Cys Ser
 435 440 445
 Asp Asn Asn Ile Thr Thr Gln Lys Val Val Gln Ser Leu Met Ser Ser
 450 455 460
 15 Gly Leu Leu Gln Pro Ala Gly Ile Ala Met Thr Leu Ser Asn Thr Gly
 465 470 475 480
 Gln Gln Trp Asp Phe Pro Asn Gly Trp Pro Pro Leu Gln His Ile Ile
 20 485 490 495
 Ile Glu Gly Leu Leu Arg Ser Gly Leu Glu Glu Ala Arg Thr Leu Ala
 500 505 510
 25 Lys Asp Ile Ala Ile Arg Trp Leu Arg Thr Asn Tyr Val Thr Tyr Lys
 515 520 525
 Lys Thr Gly Ala Met Tyr Glu Lys Tyr Asp Val Thr Lys Cys Gly Ala
 530 535 540
 30 Tyr Gly Gly Gly Gly Glu Tyr Met Ser Gln Thr Gly Phe Gly Trp Ser
 545 550 555 560
 Asn Gly Val Val Leu Ala Leu Leu Glu Glu Phe Gly Trp Pro Glu Asp
 35 565 570 575
 Leu Lys Ile Asp Cys
 580

40 (2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 45 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50 (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

CTCAGATCTG GCCACAAA

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(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

15 GTGCTCGTCT GCAGGTGC

18

CLAIMS

1. Method of modification of the development and/or composition of cells, tissue or organs *in vivo* by inducing a change in the metabolic availability of trehalose-6-phosphate.
5
2. Method for the stimulation of carbon flow in the glycolytic direction in a cell by decreasing the intracellular availability trehalose-6-phosphate.
- 10 3. Method for the inhibition of carbon flow in the glycolytic direction in a cell by increasing the intracellular availability of trehalose-6-phosphate.
4. Method for the inhibition of photosynthesis in a cell by
15 decreasing the intracellular availability of trehalose-6-phosphate.
5. Method for the stimulation of photosynthesis in a cell by increasing the intracellular availability of trehalose-6-phosphate.
- 20 6. Method for the stimulation of sink-related activity by increasing the intracellular availability of trehalose-6-phosphate.
7. Method for the stimulation of growth of a cell or tissue by decreasing the intracellular availability of trehalose-6-phosphate.
25
8. Method for the inhibition of growth of a cell or tissue by increasing the intracellular availability of trehalose-6-phosphate.
9. Method for increasing metabolism of cells by decreasing the
30 intracellular availability of trehalose-6-phosphate.
10. Method according to claim 2, 4, 7 or 9, characterized in that said decrease of the intracellular concentration of trehalose-6-phosphate is effected by an increase in trehalose-phosphate-
35 phosphatase (TPP) activity.

11. Method according claim 10, characterized in that the increase in TPP activity is achieved by transformation of said cells with a vector capable of expression of the enzyme TPP.
- 5 12. Method according to claim 11, characterized in that said cells are transformed with a vector comprising a heterologous gene encoding TPP.
13. Method according to claim 2, 4, 7 or 9, characterized in that
10 said decrease of the intracellular concentration of trehalose-6-phosphate is effected by a decrease in trehalose-phosphate synthase (TPS) activity.
14. Method according to claim 13, characterized in that said
15 decrease in TPS activity is effected by transformation of said cells with a vector capable of expression of a molecule that inhibits TPS.
15. Method according to claim 14, characterized in that said vector comprises the antisense gene of TPS.
- 20 16. Method according to claim 10, characterized in that said decrease is due to mutation of the endogenous TPP enzyme.
17. Method according to claim 10, characterized in that the
25 decrease of trehalose-6-phosphate is effected by the relative overexpression of a phospho-alpha-(1,1)-glucosidase.
18. Method according to claim 3, 5, 6 or 8, characterized in that
30 said increase of the intracellular concentration of trehalose-6-phosphate is effected by an increase in TPS activity.
19. Method according claim 18, characterized in that the increase in TPS activity is achieved by transformation of said cells with a vector capable of expression of the enzyme TPS.
- 35 20. Method according to claim 19, characterized in that said cells are transformed with a vector comprising a heterologous gene encoding TPS.

21. Method according to claim 3, 5, 6 or 8, characterized in that said increase of the intracellular concentration of trehalose-6-phosphate is effected by a decrease in TPP activity.
- 5 22. Method according to claim 21, characterized in that said decrease in TPP activity is effected by transformation of said cells with a vector capable of expression of a molecule that inhibits TPP.
23. Method according to claim 22, characterized in that said vector
10 comprises the antisense gene of TPS.
24. Method according to claim 18, characterized in that said increase is due to a mutation of the endogenous TPS enzyme.
- 15 25. Method according to any one of claims 1-24, characterized in that said cell or cells are located in a plant.
26. Method according to claim 25, characterized in that said plant is a transgenic plant.
- 20 27. Method according to claim 26, characterized in that said transgenic plant is produced by transformation with *Agrobacterium tumefaciens*.
- 25 28. Method according to any one of claims 1-24, characterized in that said cell or cells are located in an animal, preferably a mammal, more preferably a human being.
29. Method according to any one of claims 1-24, characterized in
30 that said cells are microorganisms, preferably a microorganism selected from the group consisting of bacteria, microbes, yeasts, fungi, cell cultures, oocytes, sperm cells, hybridomas, Protista and callus.
- 35 30. A cloning vector which comprises a gene coding for TPP.

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31. The cloning vector of claim 30, characterized in that it comprises a nucleotide sequence selected from the group of nucleotide sequences depicted in SEQ ID NO: 3, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 17 and the parts coding for TPP from the
5 bipartite enzymes as coded by SEQ ID NO: 24, SEQ ID NO: 28, SEQ ID NO: 39, SEQ ID NO: 42 and SEQ ID NO: 44.
32. A cloning vector which comprises an antisense gene for TPS, which upon expression is able to prevent functional activity of the
10 endogenous TPS gene.
33. A cloning vector which comprises a gene for TPS, characterized in that it comprises a nucleotide sequence selected from the group of nucleotide sequences depicted in SEQ ID NO: 1, SEQ ID NO: 10, SEQ ID
15 NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52 and SEQ ID NO: 53.
- 20
34. A cloning vector which comprises an antisense gene for TPP, which upon expression is able to prevent functional activity of the endogenous TPP gene.
- 25 35. Plant characterized in that it or one of its ancestors is transformed with a vector comprising the nucleotide sequence coding for TPP.
36. Plant characterized in that it or one of its ancestors is
30 transformed with a vector comprising the nucleotide sequence coding for an antisense gene of TPP.
37. Plant characterized in that it or one of its ancestors is transformed with a vector comprising the nucleotide sequence coding
35 for an antisense gene of TPS.
38. Use of trehalose-6-phosphate to influence carbohydrate partitioning in cells.

39. Use of trehalose-6-phosphate to increase biomass.
40. Use of trehalose-6-phosphate to affect hexokinase activity.
- 5 41. Use of trehalose-6-phosphate to affect hexokinase signalling function.
42. Use of trehalose-6-phosphate to affect cell wall synthesis.
- 10 43. Use of compounds influencing the intracellular availability of trehalose-6-phosphate to increase biomass.
44. Use of compounds influencing the intracellular availability of trehalose-6-phosphate to affect hexokinase activity.
- 15 45. Use of compounds influencing the intracellular availability of trehalose-6-phosphate to affect photosynthesis.
46. Use of compounds influencing the intracellular availability of trehalose-6-phosphate to affect the carbon flow in the glycolytic pathway.
- 20 47. Method for the prevention of cold sweetening by increasing the intracellular availability of trehalose-6-phosphate.
- 25 48. Method for the inhibition of invertase in beet after harvest by increasing the intracellular availability of trehalose-6-phosphate.
49. Use of compounds influencing the intracellular availability of trehalose-6-phosphate to affect cold sweetening or invertase inhibition.
- 30 50. Method according to claim 47 or 48, characterized in that increasing the intracellular availability of T-6-P results from the
- 35 increase of trehalose phosphate synthase activity.

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51. Method according to claim 47, characterized in that the regulation of the availability of T-6-P is specifically altered in potato tubers.
- 5 52. Method according to claim 51, characterized in that a gene coding for trehalose phosphate synthase is specifically expressed in tubers.
53. Method according to claim 52, characterized in that said gene
10 is the TPS gene from *Escherichia coli*.
54. Method according to claim 48, characterized in that the regulation of the availability of T-6-P is specifically altered in beet taproots.
- 15 55. Method according to claim 54, characterized in that a gene coding for trehalose phosphate synthase is specifically expressed in taproots.
- 20 56. Method for the accumulation of trehalose, characterized in that an organism is transformed with a DNA sequence coding for a bipartite TPS-TPP enzyme.
57. Method according to claim 56, characterized in that said gene
25 is the bipartite gene from *Arabidopsis thaliana*.
58. Method according to claim 56, characterized in that said gene is the bipartite gene from *Selaginella lepidophylla*.
- 30 59. Method according to claim 56, characterized in that said gene is the human bipartite gene.
60. Method according to claim 56, characterized in that said gene is the bipartite gene from *Helianthus annuus*.
- 35 61. Method to prevent metabolic steering during the production of trehalose by expression of a DNA sequence coding for a bipartite TPS-TPP enzyme.

62. Method according to claims 1-24, characterized in that expression of TPP or TPS is limited to a specific tissue.
63. Method according to claims 1-24, characterized in that
5 expression of TPP or TPS is under control of an inducible promoter.
64. Method of modification of the development and/or composition of cells, tissue or organs *in vivo* by inducing a change in the metabolic availability of trehalase.
10
65. Method for the stimulation of carbon flow in the glycolytic direction in a cell by increasing the intracellular availability of trehalase.
- 15 66. Method for the inhibition of carbon flow in the glycolytic direction in a cell by decreasing the intracellular availability of trehalase.
67. Method for the stimulation of sink-related activity by
20 decreasing the intracellular availability of trehalase.
68. Method for the stimulation of growth of a cell or tissue by increasing the intracellular availability of trehalase.
- 25 69. Method for the inhibition of growth of a cell or tissue by decreasing the intracellular availability of trehalase.
70. Method for increasing metabolism of cells by increasing the intracellular availability of trehalase.
30
71. Method for the stimulation of carbon flow in the glycolytic direction in a cell by expression of trehalose-6-phosphate phosphatase.
- 35 72. Method for the inhibition of carbon flow in the glycolytic direction in a cell by expression of trehalose-6-phosphate synthase.

73. Method for the inhibition of photosynthesis in a cell by expression of trehalose-6-phosphate phosphatase.
74. Method for the stimulation of photosynthesis in a cell by
5 expression of trehalose-6-phosphate synthase.
75. Method for the stimulation of sink-related activity by expression of trehalose-6-phosphate synthase.
- 10 76. Method for the stimulation of growth of a cell or tissue by expression of trehalose-6-phosphate phosphatase.
77. Method for the inhibition of growth of a cell or tissue by expression of trehalose-6-phosphate synthase.
- 15 78. Method for increasing metabolism of cells by expression of trehalose-6-phosphate phosphatase.
79. Method for the prevention of cold sweetening by expression of
20 trehalose-6-phosphate synthase.
80. Method for the prevention of cold sweetening by decreasing the availability of intracellular trehalase.
- 25 81. Method for the prevention of bolting by decreasing the intracellular availability of trehalose-6-phosphate.
82. Method for the prevention of bolting by expression of trehalose-6-phosphate phosphatase.
- 30 83. Method for the induction of bolting by increasing the intracellular availability of trehalose-6-phosphate.
84. Method for the induction of bolting by expression of trehalose-
35 6-phosphate synthase.
85. Method for the induction of bolting by decreasing the intracellular availability of trehalase.

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86. Method for increasing the yield of plants by transforming them with an enzyme coding for trehalose-6-phosphate phosphatase.
87. Method for increasing the yield of plants by increasing the intracellular availability of trehalose-6-phosphate.
88. Polynucleotide coding for trehalose-6-phosphate synthase, characterized in that it is a bipartite enzyme which has a mutation in the part coding for trehalose-6-phosphate phosphatase.
89. Polynucleotide coding for trehalose-6-phosphate phosphatase, characterized in that it is a bipartite enzyme which has a mutation in the part coding for trehalose-6-phosphate synthase.
90. Polynucleotide according to claim 88 or 89, characterized in that the bipartite gene is human TPS/TPP.
91. Polynucleotide according to claim 90, characterized in that the human TPS/TPP has an amino acid sequence according to SEQ ID NO: 11.
92. Polynucleotide according to claim 91, characterized in that it comprises the polynucleotide sequence of SEQ ID NO:10.
93. Polynucleotide according to claim 88 or 89, characterized in that the bipartite gene is *Arabidopsis thaliana* TPS/TPP.
94. Polynucleotide according to claim 93, characterized in that the human TPS/TPP has an amino acid sequence according to SEQ ID NO: 40.
95. Polynucleotide according to claim 94, characterized in that it comprises the polynucleotide sequence of SEQ ID NO:39.
96. Polynucleotide according to claim 88 or 89, characterized in that the bipartite gene is *Selaginella lepidophylla* TPS/TPP.
97. Polynucleotide according to claim 96, characterized in that the human TPS/TPP comprises an amino acid sequence according to SEQ ID NO: 43 or a mutein thereof.

98. Polynucleotide according to claim 97, characterized in that it comprises the polynucleotide sequence of SEQ ID NO:42 or SEQ ID NO:44.
99. Polynucleotide according to claim 88 or 89, characterized in
5 that the bipartite gene is *Helianthus annuus* TPS/TPP.
100. Polynucleotide according to claim 99, characterized in that the human TPS/TPP comprises an amino acid sequence according to SEQ ID NO: 25 or a mutein thereof.
- 10
101. Polynucleotide according to claim 100, characterized in that it comprises the polynucleotide sequence of SEQ ID NO:24 or SEQ ID NO:26 or SEQ ID NO:28.
- 15 102. Vector harbouring a polynucleotide according to any of claims 88 to 101.
103. Host organism comprising a vector according to claim 102.
- 20 104. Host organism according to claim 103, characterized in that it is *Agrobacterium tumefaciens*.
105. Cell transformed with a host organism according to claim 103 or 104.
- 25 106. Cell according to claim 105, characterized in that it is a plant cell.
107. Plant or plant part, regenerated from the plant cell according
30 to claim 106.

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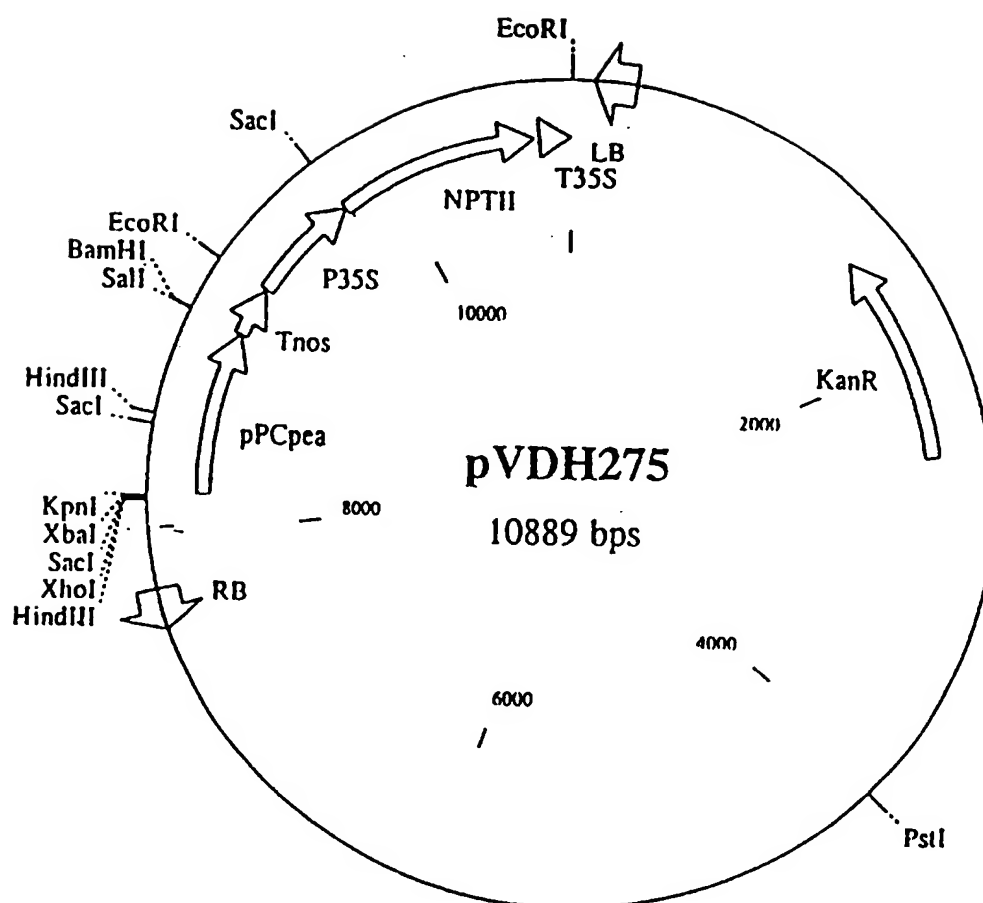


Fig. 1

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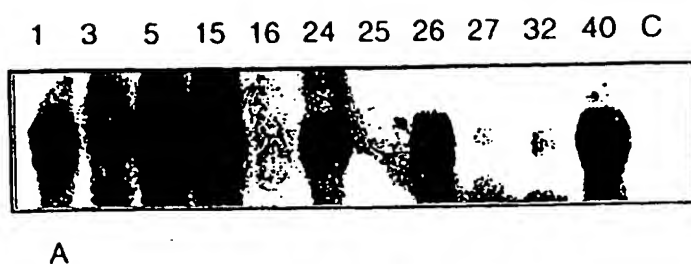


Fig. 2

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LINEUP of: TPSPLANT from: 1 to: 595 April 19, 1996 14:01 ..

	1	50
Tpsyest	MTTDNAKAQL TSSSGGNIIV VSNRLPVTIT KNSSTGQY EY AMSSGGLVTA	
	51	100
Tpsyest	LEGLKKTYTF KWFGWPGLEI PDDEKDQVRK DLLEKFNAVP IFLSDEIADL	
	101	150
Tpssel8		
Tpsyest	HYNGFSNSIL WPLFHYHPGE INF DENAWLA YNEANQTFTN EIAKTMNHND	
	151	200
Tpsrice2		YRSLPVR
Tpssun10		GWFLHTPFPS SEVYKTLPMR
Tpssel43		GWFLHTPFPS SEIYRTLPLR
Tpssel8	IMWVHDYHLC LVPQMIRQKL PDVQI.....	GFPLHTAFPS SEVFRCLAAR
Tpsyest	LIWVHDYHLM LVPEMLRVKI HEKQLQNVKV	GWFLHTPFPS SEIYRILPVR
	201	250
Tpsrice2	DEILKSLNC DLIGFHTFDY ARHFLSCCSR MLGIEYQSKR	GYIGLDYFGR
Tpssun10	NELLKGLNA DLIGFHTYDY ARHFLTCCSR MFGLDHQLKR	GYIFLEYNGR
Tpssel43	AELLQGVLGA DLVGFHTYDY ARHFVS.....	..AMHTDTRA GRHSQGVEDQ
Tpssel8	KELLDGMLGA NLVAFQTPEY AHFLQ.....	..XVQSHXSL LKQPR.....
Tpsyest	QEILKGVLSCLVGFHTYDY ARHFLSSVQR	VLNVNTLPNG VEYQGRFVN
	251	300
Tpsrice2	TVGIKIMPVG INMTQLQTQI RLPDLEWRVA NSGSSLMGRL	SCSVWMIWTY
Ricetps	EWRV SELQQQPEGK	TVLLGVDDM
Tpssun10	SIEIKIKASG IHVGRMESYL SQPDTRLQVQ	EVK..KEIVLLGVDDL
Tpssel43	GKITRVAA.. FVDRFGAIY RRVEDAVKK HMQELSQVLL	S*GYVGVDRL
Tpssel8KA.. FS.XRFVNVW ...SX..MQE	ALRXVKKVIV ARDKLTTSR.
Tpsyest	GAPPIGIDVD KFTDGLKKES VQKRIQQLKE	TFKGCKIIV.GVDRL
	301	350
Tpsatal3		.G
Tpsatal56	N EELRGKVVLV QITNPARSS.G
Tpsrice2	LR..GLI*KF LRFEQMLRTH PKWQPRQFWC	RFKPRVVVGR TLXYSXDXV
Ricetps	DIFKGINLKL LAFENMLRTH PKWQGRAVLV	QIANPARGK.G
Tpssun10	DIFKGVNFKV LALEKLLKSH PSWQGRVVLV	QILNPSR.R.C
Tpssel43	DMIKGIPQKL LAFEKFLEEN SEWRDKVVLV	QIAPVTRTD.V
Tpssel8VREKL LSYELFLNKN PQWRDKVVLV	QVATSTTEDS ELAATXYPKL
Tpsyest	DYIKGVPQKL HAMEVFLNEH PEWRGKVVLV	QVAVPSRGDV EEYQYLRSVV

Fig. 3 (part 1)

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351                                     400
Tpsatal3 IDVEEIRGEI EESCRRING. EFGKXGYQPI IYIDXPVSIN EINAYXHIAE
Tpsatal56 KDVQDVEKQI NXIADEINSK FGRPGGYKPI VFVNGPVSTL DKVAYYAISE
Tpsrice2 QXVMTFQAGI SL
Ricetps KDLEAIQAEI HESCKRING. EFGQSGYSPV VFIDRDVSSV EEDCLLHNSR
Tpssun10 QDVDEINAEI RTVCERINN. ELGSPGYQPV VLIDGPVSLs EKAAYYVIAD
Tpssel43 LEYQKLTSQV HEIVGRING. RFGSLTAVPI HHLDRSMKFP ELCALYAITD
Tpssel18 LHVLTLCRR SHTPTRLPQ. ARHCVLAVPR TSLDRRCSCN QLF.....
Tpsyeast NELVG..... RING. QFGTVEFVPI HFMHKSIPFE ELISLYAVSD

401                                     450
Tpsatal3 CVVVTAVRDG MNLTPYEYIV CRQGLLGSES DFSGPKKSML ....VASXFI
Tpsatal56 CVVVNXVRDG MNLVPYKYTV TRQGSPALDA ALGFGEDDVR KSVIIVSEFI
Tpsatal142 AVVDSSPR TSTLVVSEFI
Tpsrice3 GPK KSMLVVSEFI
Ricetps MCGGDCC*GW D*LDTIWIYC L*GRGLTXHQ R
Tpssun10 MAIVTPLRDG MNLV
Tpssel43 VLLVTSLRDG MNFV
Tpssel18 .....DG MNLV
Tpsyeast VCLVSSTRDG MNLVSYEYIA CQEE..... K KGSLILSEFT

451                                     500
Tpsatal3 WMXPFRLXGA IRVNPW
Tpsatal56 GCXP.SLSGA IXVNPWNIXA V
Tpsatal142 GCSP.SLSGA IRVNPWDVDA VAEAVNSALK MSETEKQLRH EKHYHYISTH
Tpsrice3 GCSP.SLSGA IRVNPWNIEA TAEALNEAIS MSERXKQLRH EKHYRYVSTH
Tpsyeast GAAQ.SLNGA IIVNPWNTDD LSDAINEALT LPDVKKEVNW EKLYKYISKY

501                                     550
Tpsatal142 DVGWYAKSFM QDLERACRDH YSKRCWGIGF GLGFRVLSLSP SFRKLS
Tpsrice3 DVAYWSKSFV QDLERACKDH FRKPCWGIGX GFRXR
Tpsyeast TSAFWGENFV HELYSTSSSS TSSSATKN** TRCK*DDRFL LVRFSLPSLL

551                                     595
Tpsyeast FTFFILYIKL YK*HN*NATR PLLFVNACL* RC*LKLK*F FHRIG

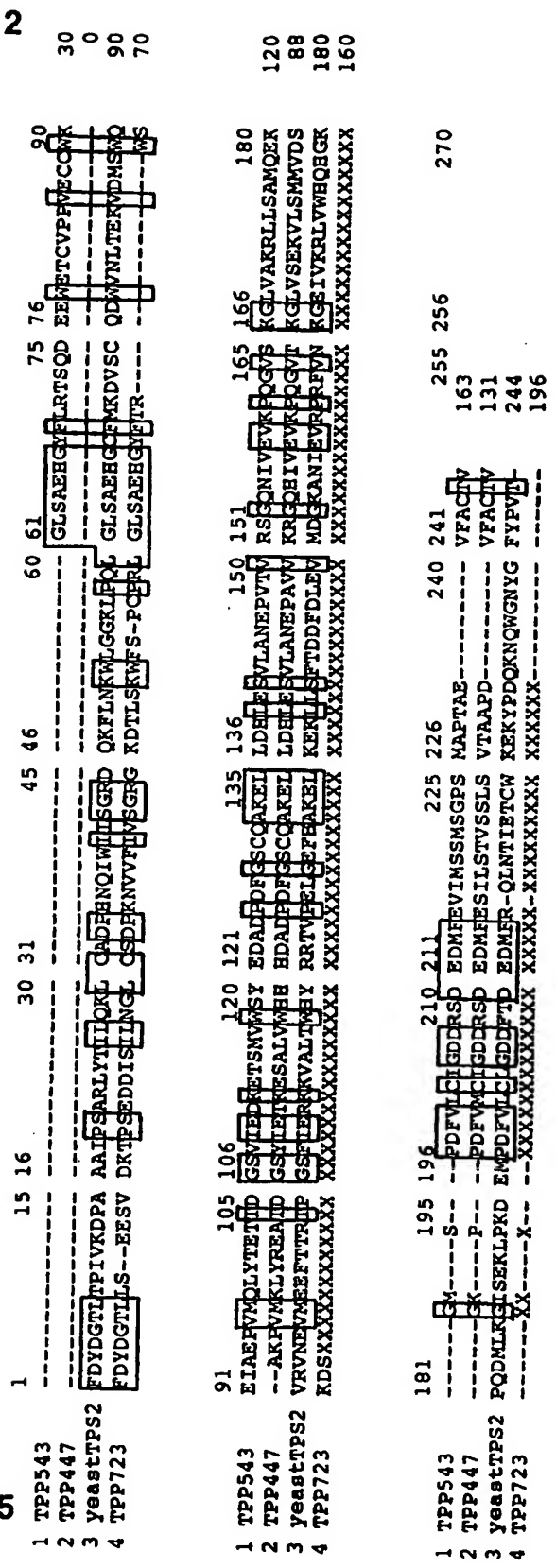
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Fig. 3 (part 2)

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Alignment tobacco TPP genes with yeast TPS2

Fig. 5



Alignment sunflower bipartite with yeast TPS2

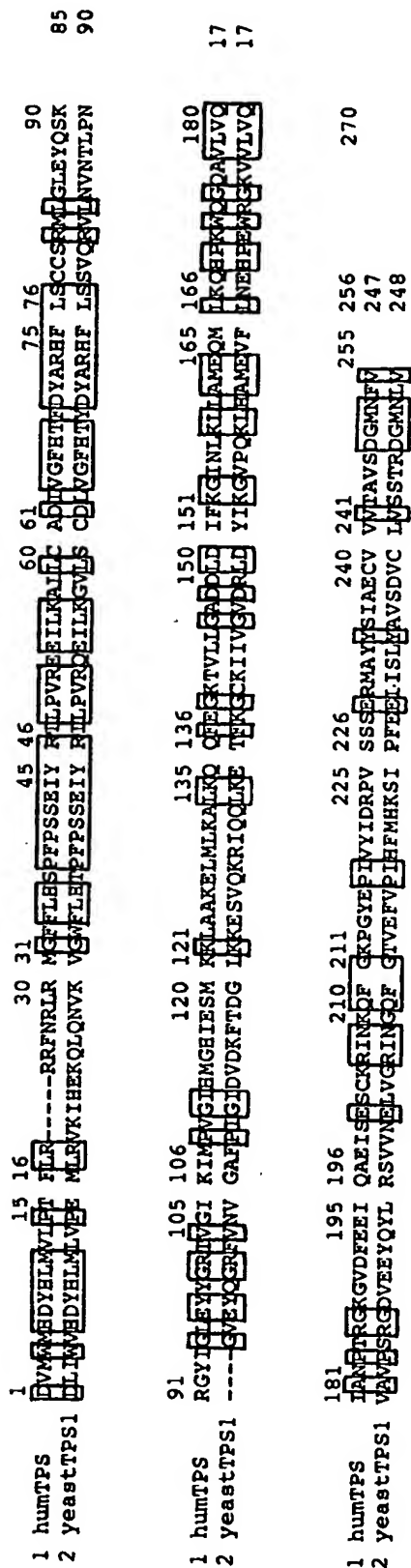
1	15	16	30	31	45	46	60	61	75	76	90
1 bipsunfl	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	27
2 yeastTPS2	MTTAAQNSPKRQR	IINCVTOLPKIQLG	ESNDOKISATTGNS	ALYSSLEYLQFDSTE	YEQ	YVVGWTGEITRT	ERNLFTFAKEKEDD	-----	-----	-----	90
1 bipsunfl	105	106	120	121	135	136	150	151	165	166	180
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	88
1 bipsunfl	181	195	196	210	211	225	226	240	241	255	256
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	176
1 bipsunfl	271	285	286	300	301	315	316	330	331	345	346
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	260
1 bipsunfl	361	375	376	390	391	405	406	420	421	435	436
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	356
1 bipsunfl	451	465	466	480	481	495	496	510	511	525	526
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	349
1 bipsunfl	541	555	556	570	571	585	586	600	601	615	616
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	446
1 bipsunfl	631	645	646	660	661	675	676	690	691	705	706
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	525
1 bipsunfl	721	735	736	750	751	765	766	780	781	795	796
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	606
1 bipsunfl	811	825	826	840	841	855	856	870	871	885	886
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	614
1 bipsunfl	901	915	916	930	931	945	946	960	961	975	976
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	696
1 bipsunfl	991	1005	1006	1020	1021	1035	1036	1050	1051	1065	1066
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	704
1 bipsunfl	1101	1115	1116	1130	1131	1145	1146	1160	1161	1175	1176
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	786
1 bipsunfl	1201	1215	1216	1230	1231	1245	1246	1260	1261	1275	1276
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	766
1 bipsunfl	1301	1315	1316	1330	1331	1345	1346	1360	1361	1375	1376
2 yeastTPS2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	876

Fig. 6

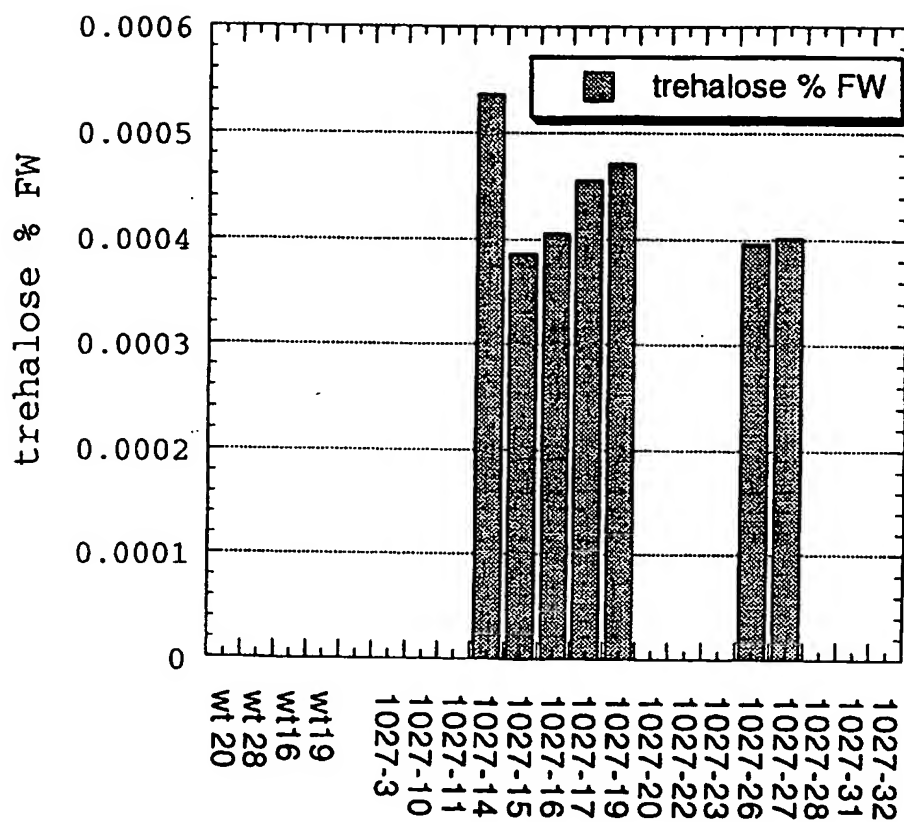
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Alignment human TPS gene with yeast TPS1

Fig. 8



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S. tuberosum pMOG1027**Fig. 9**

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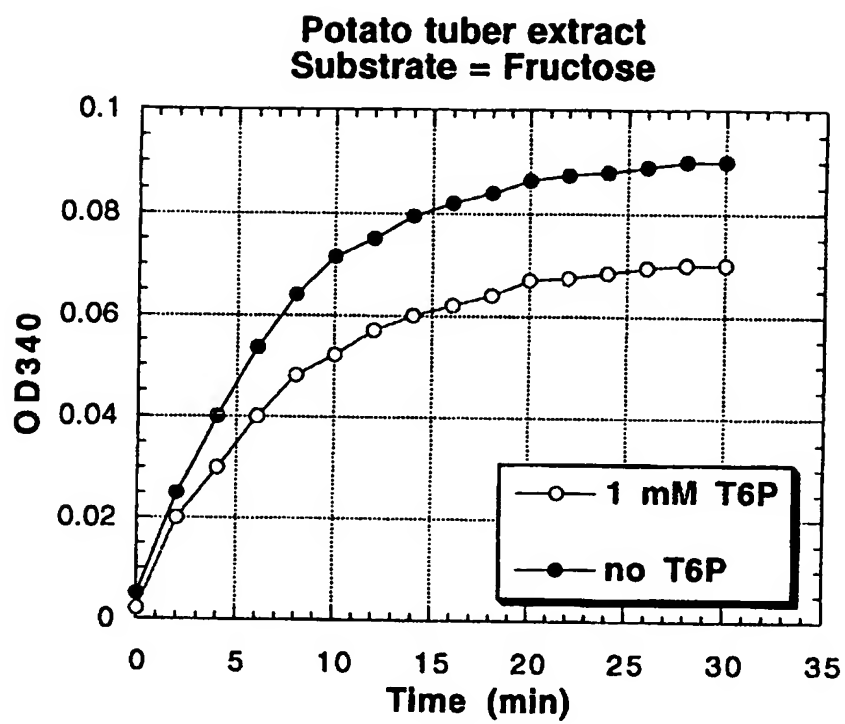
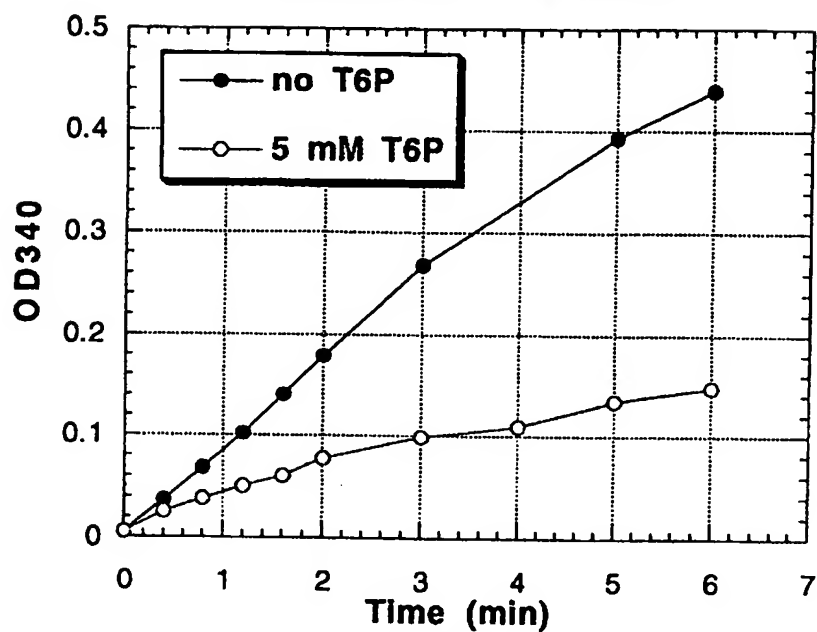


Fig. 10

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Potato tuber extract
Substrate = Fructose



Potato tuber extract
Substrate = Glucose

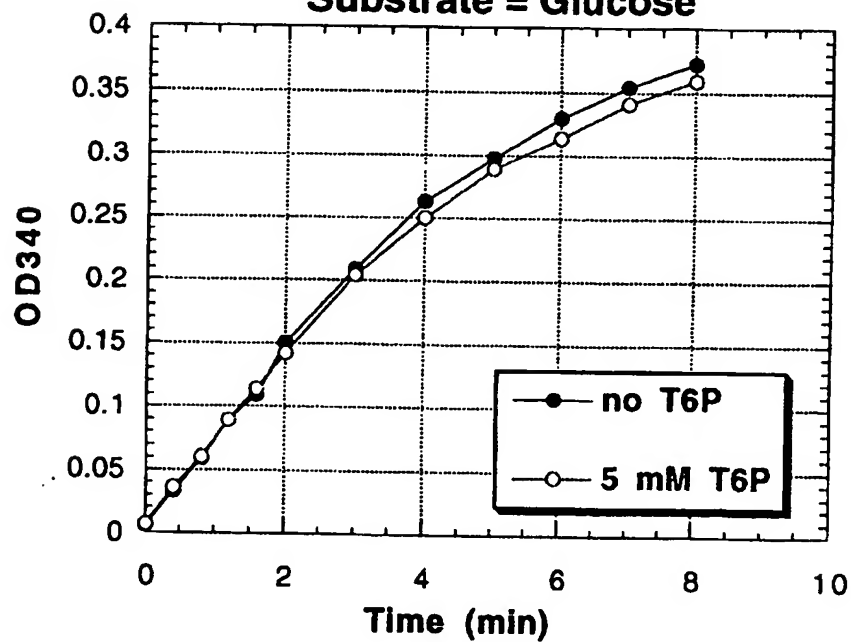


Fig. 11

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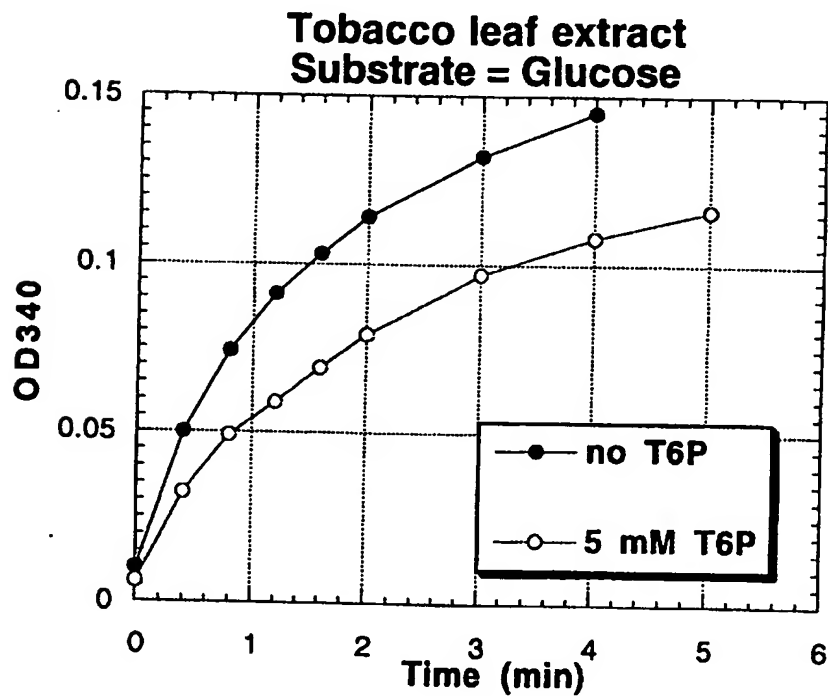
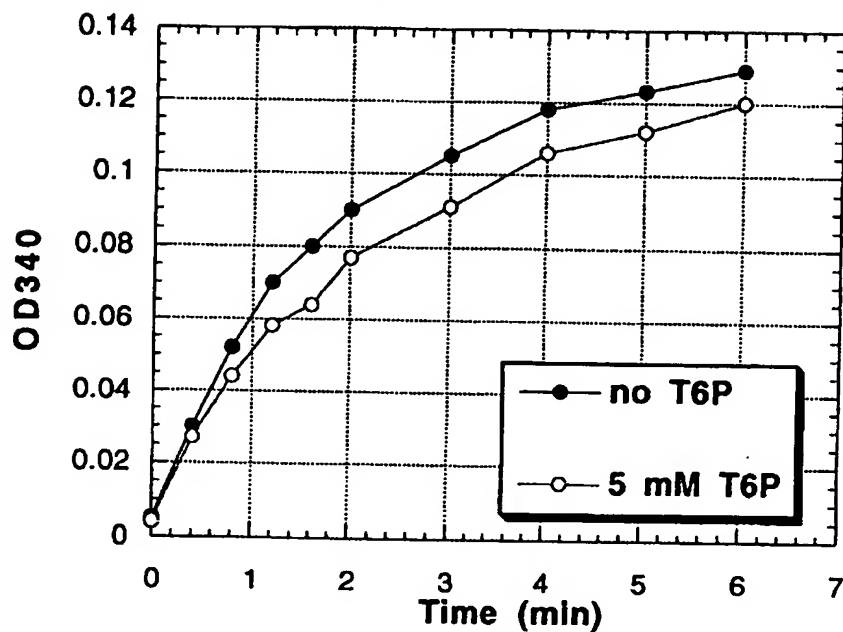
**Tobacco leaf extract
Substrate = Fructose**

Fig. 12

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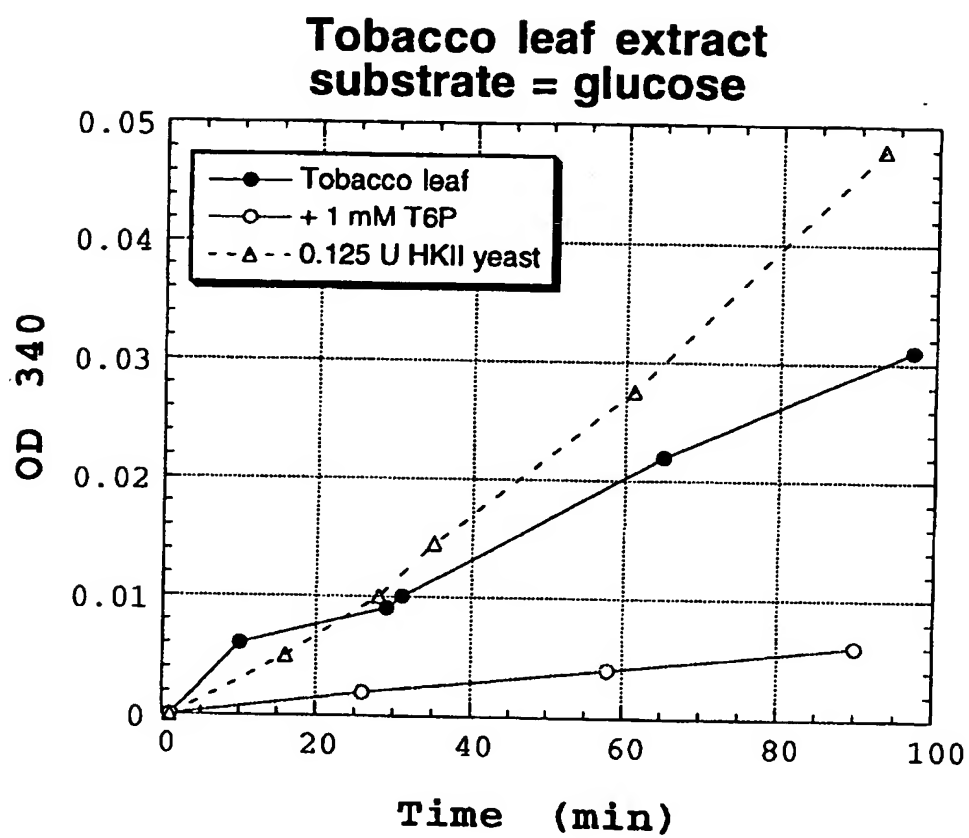
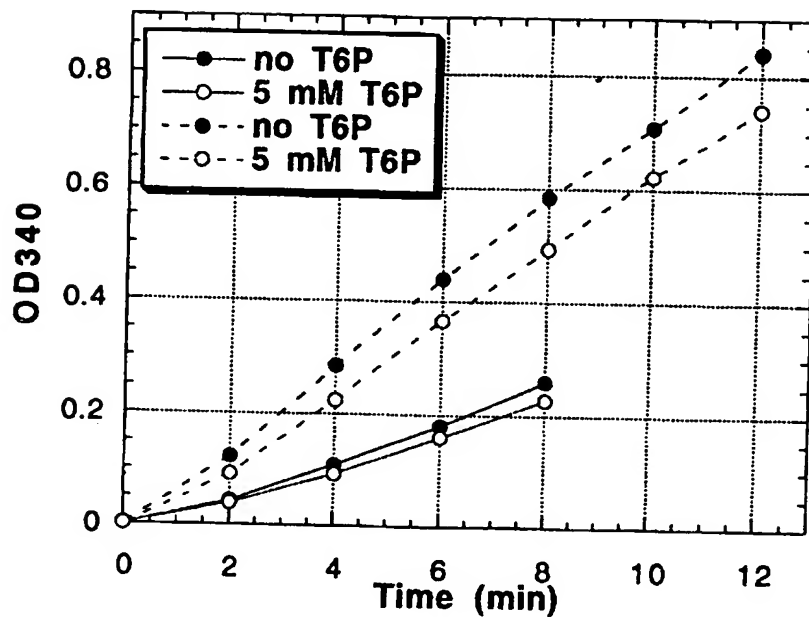


Fig. 13

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Rice leaf extract
Substrate = Fructose



Rice leaf extract
Substrate = Glucose

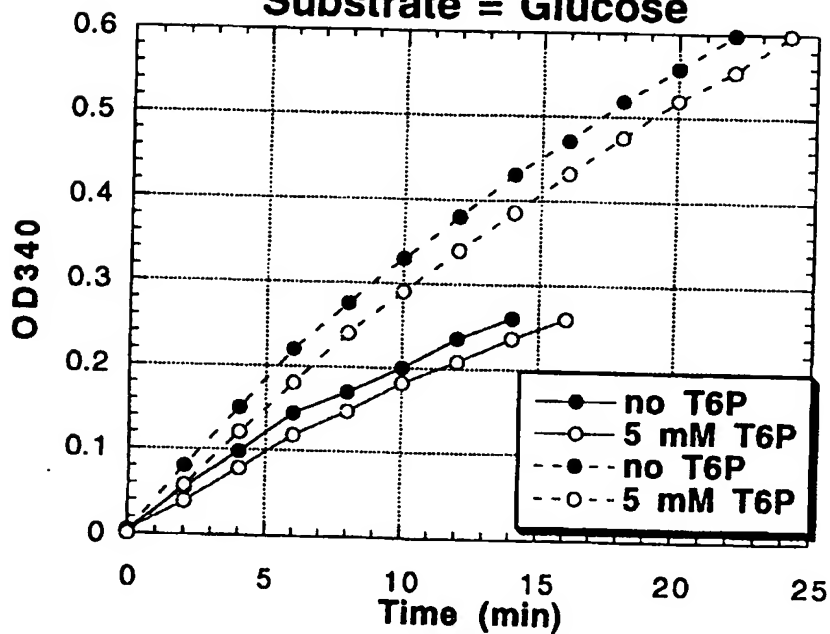
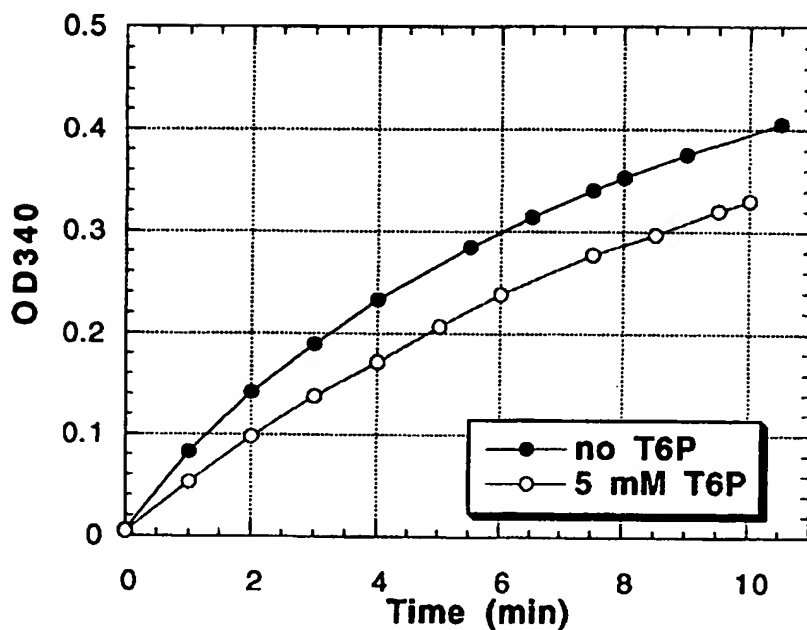


Fig. 14

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Maize leaf extract
Substrate = Fructose



Maize leaf extract
Substrate = Glucose

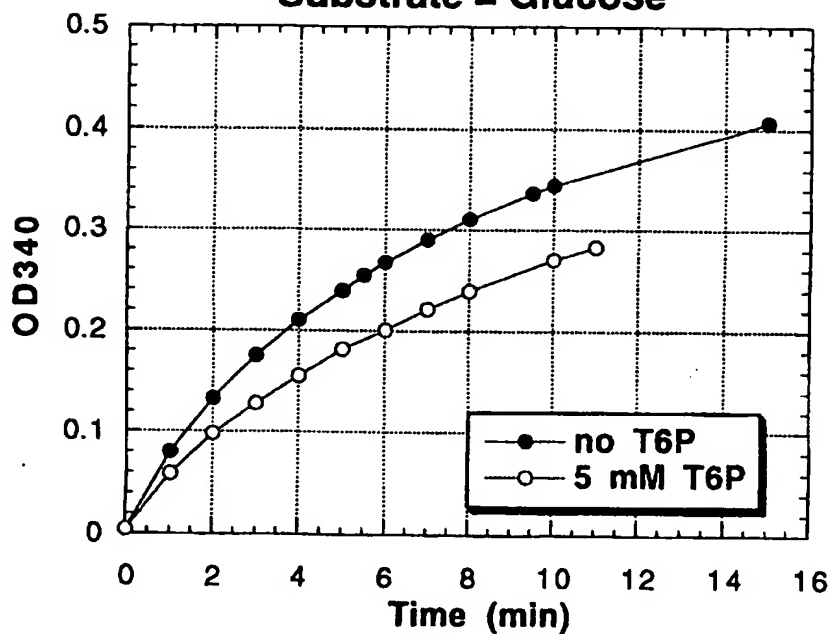


Fig. 15

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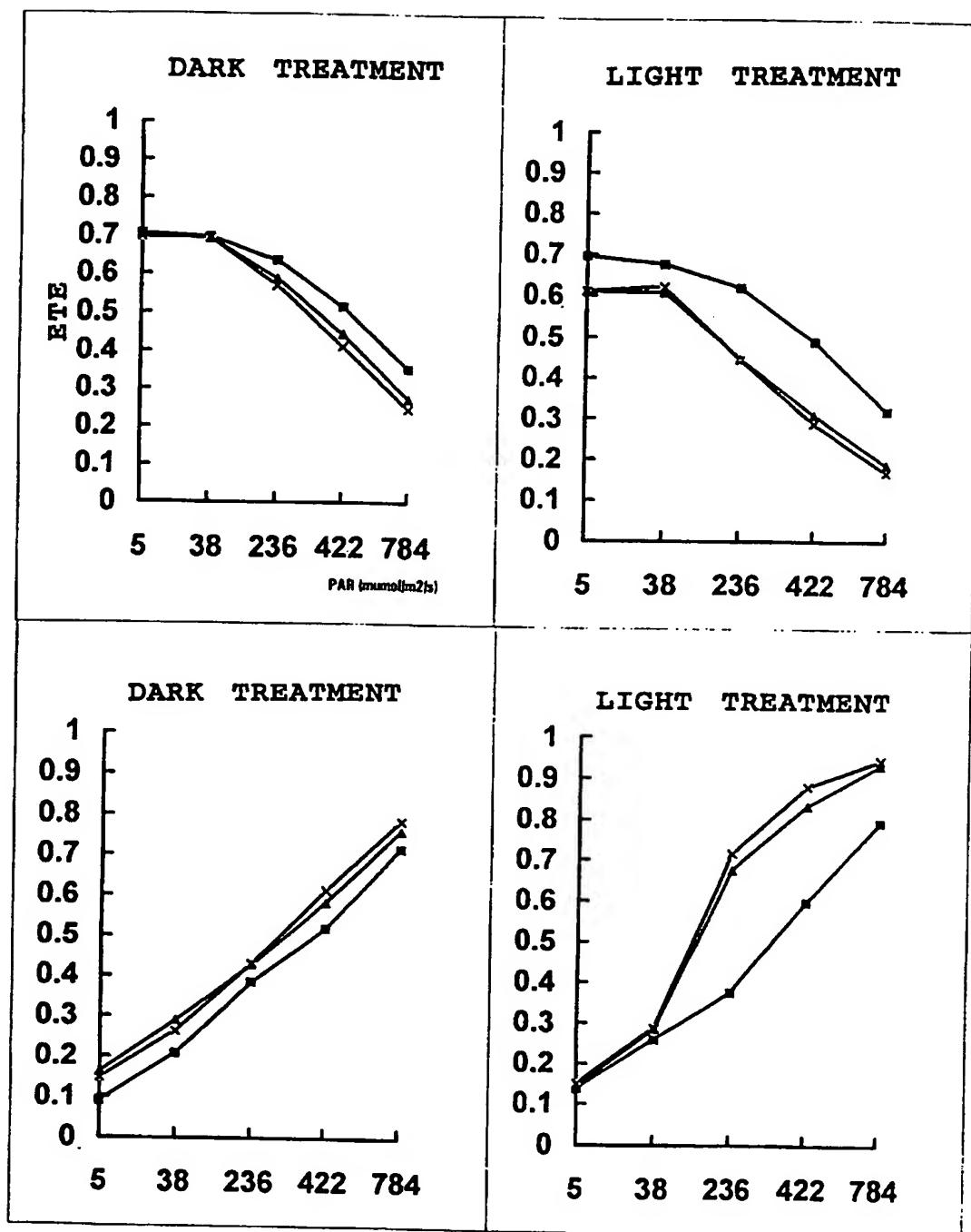


Fig. 16

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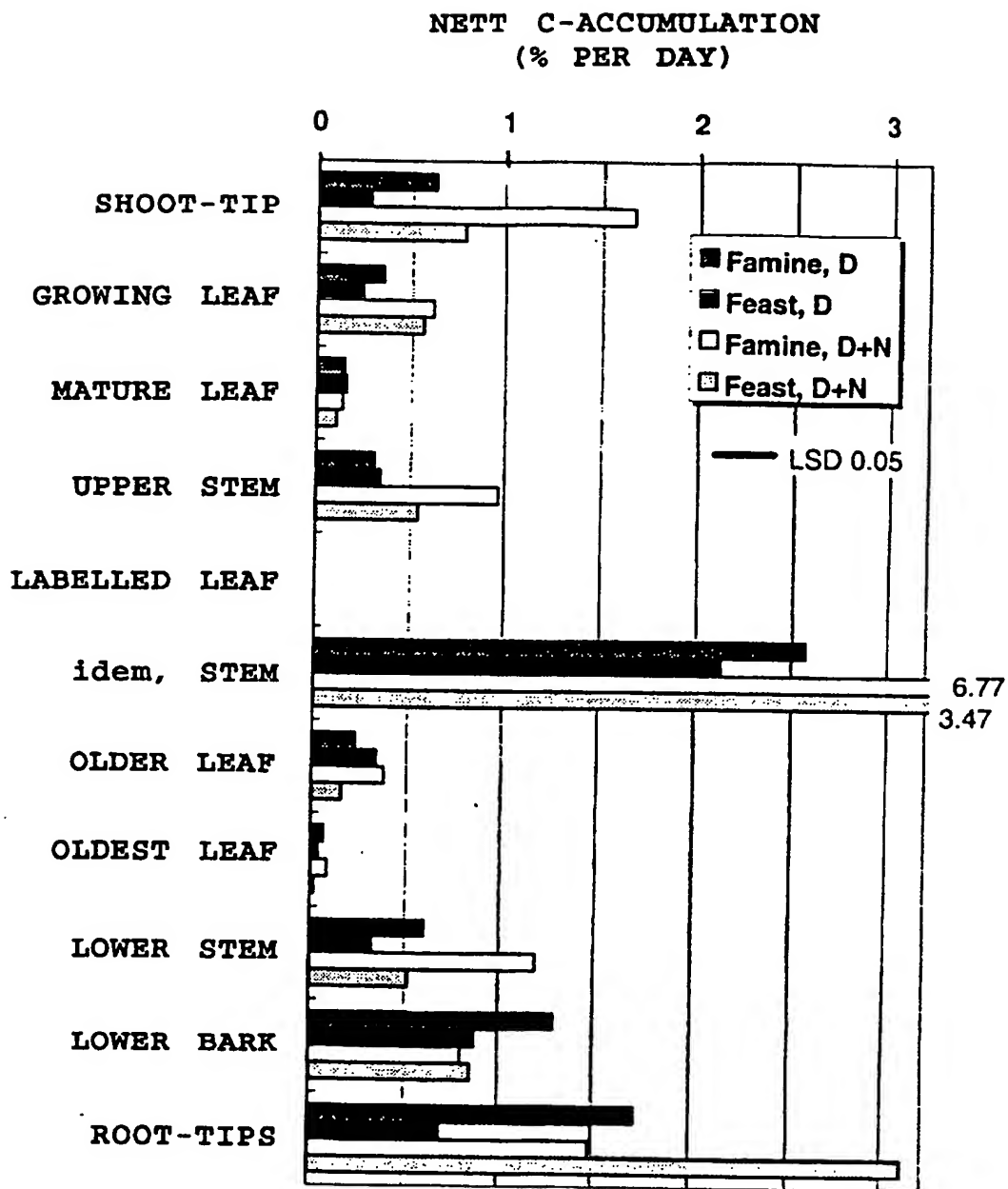


Fig. 17

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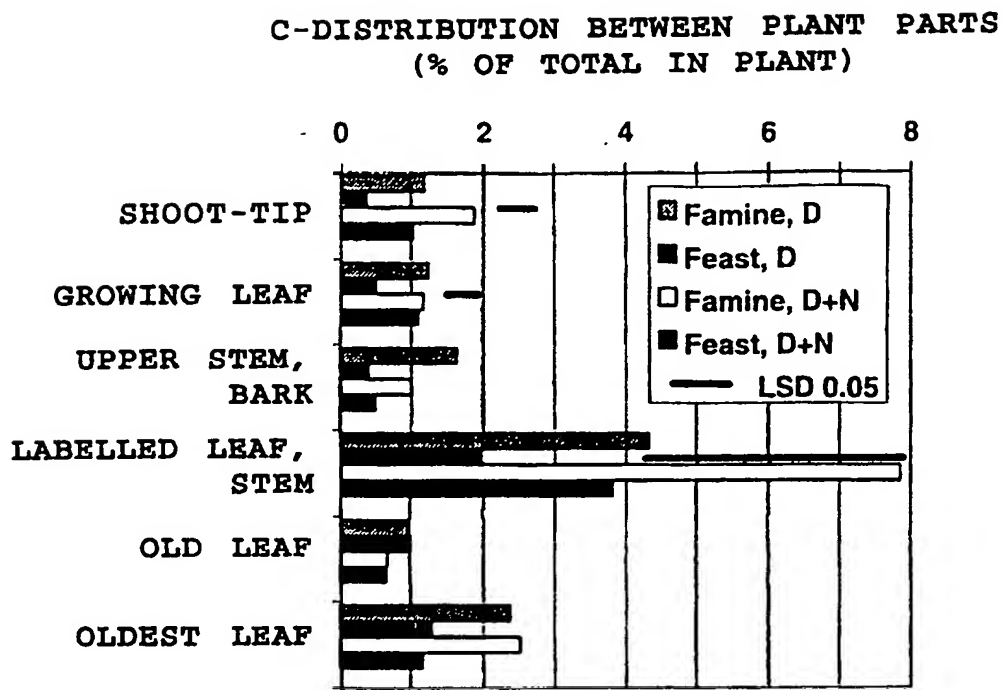


Fig. 18

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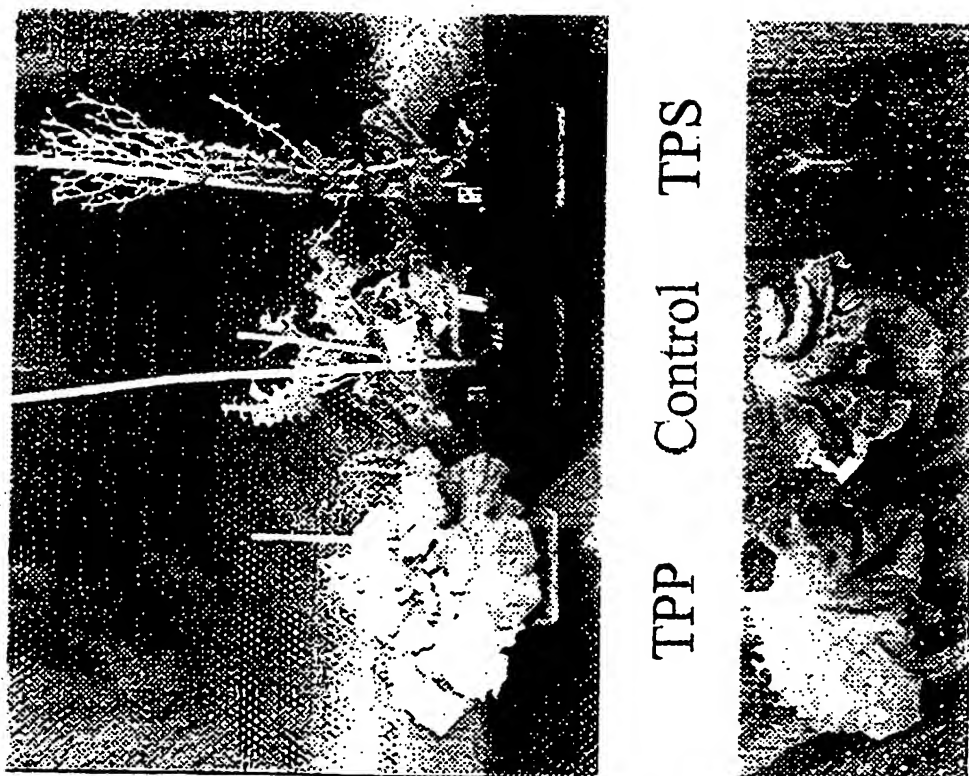
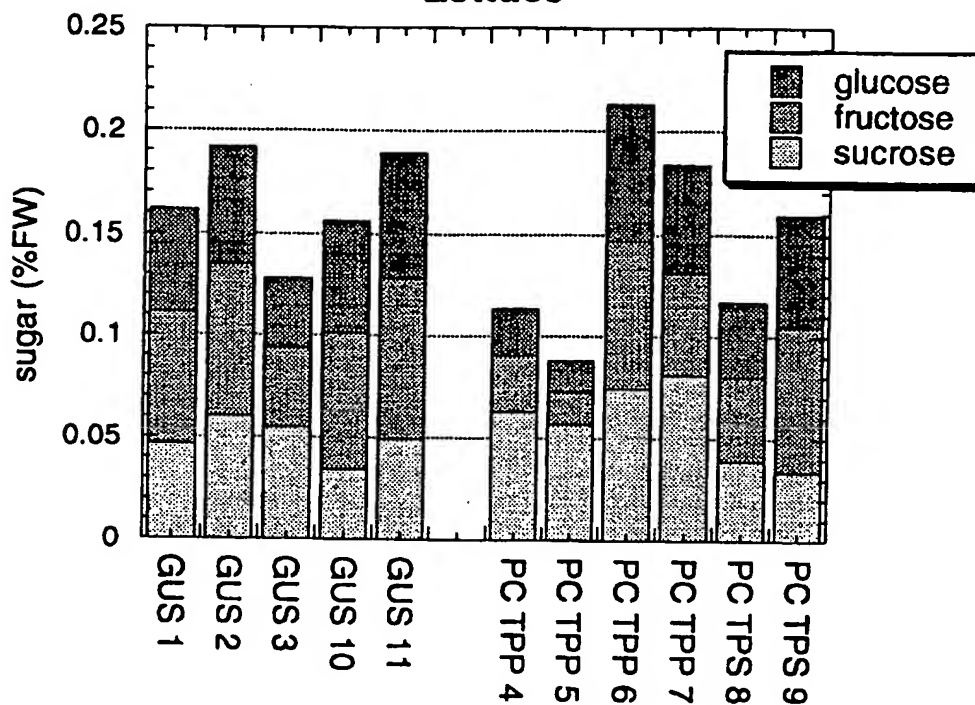


Fig. 19

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Lettuce



Beet

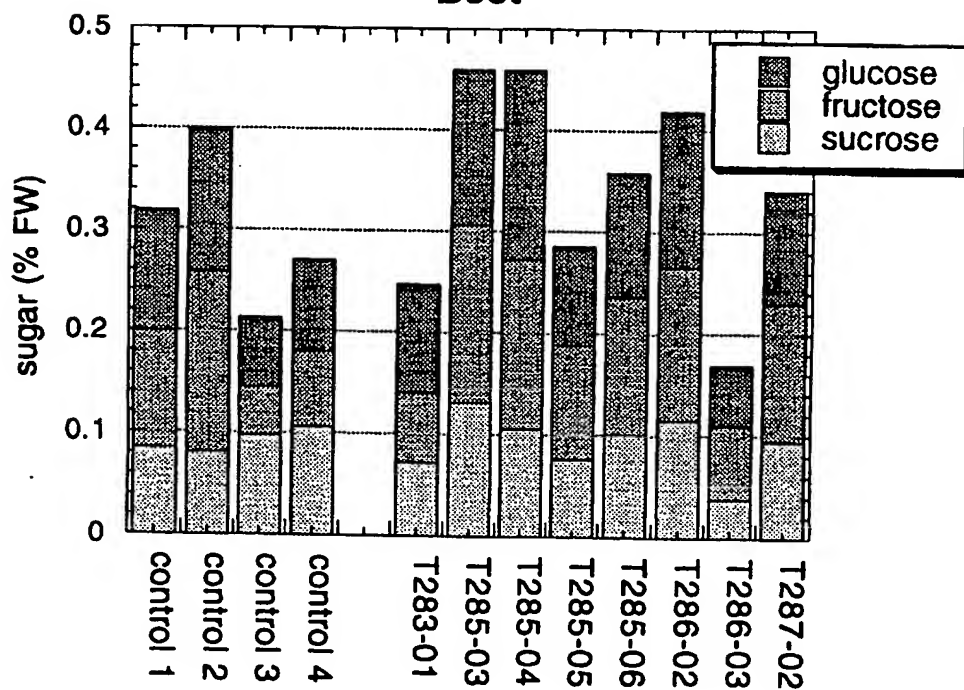
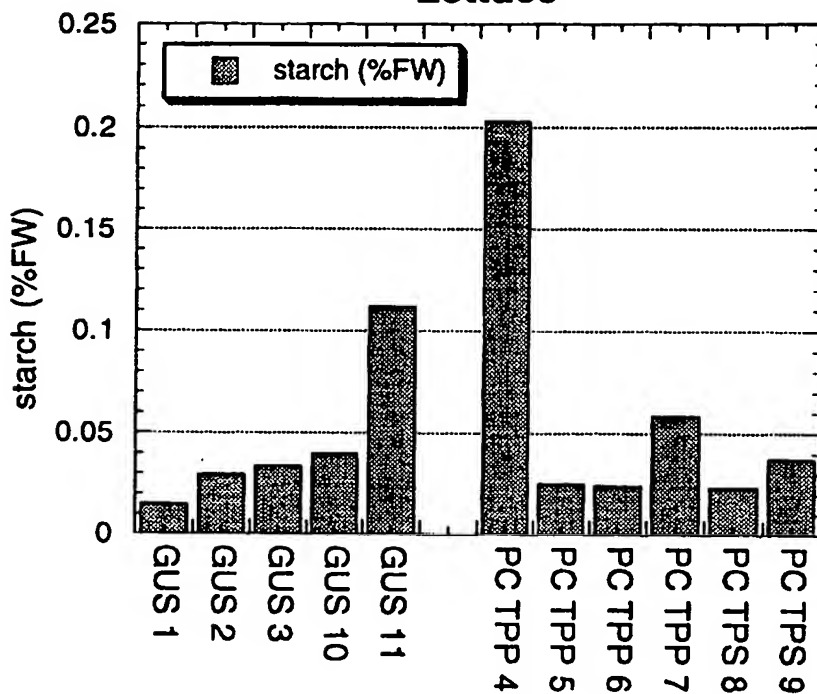
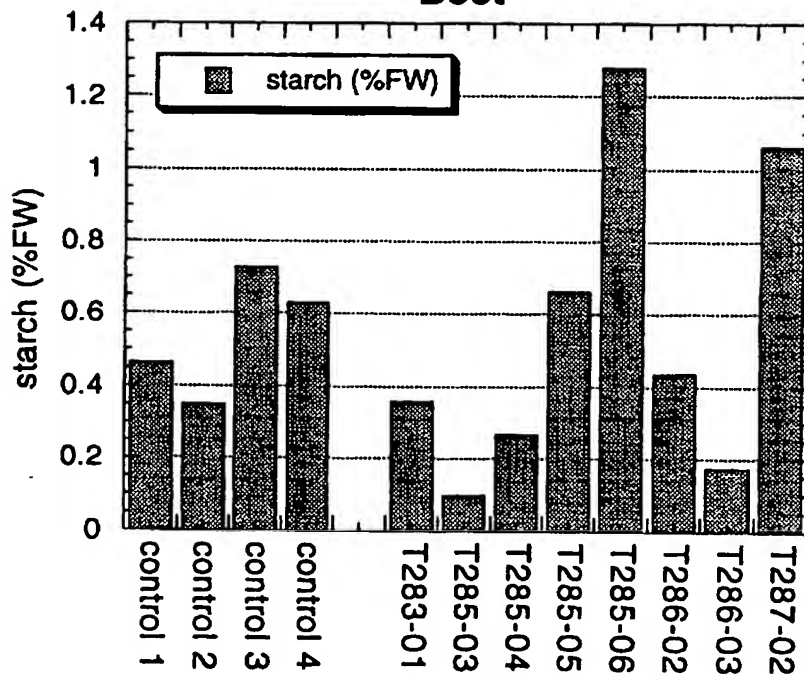


Fig. 20-1

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Lettuce**Beet****Fig. 20-2**

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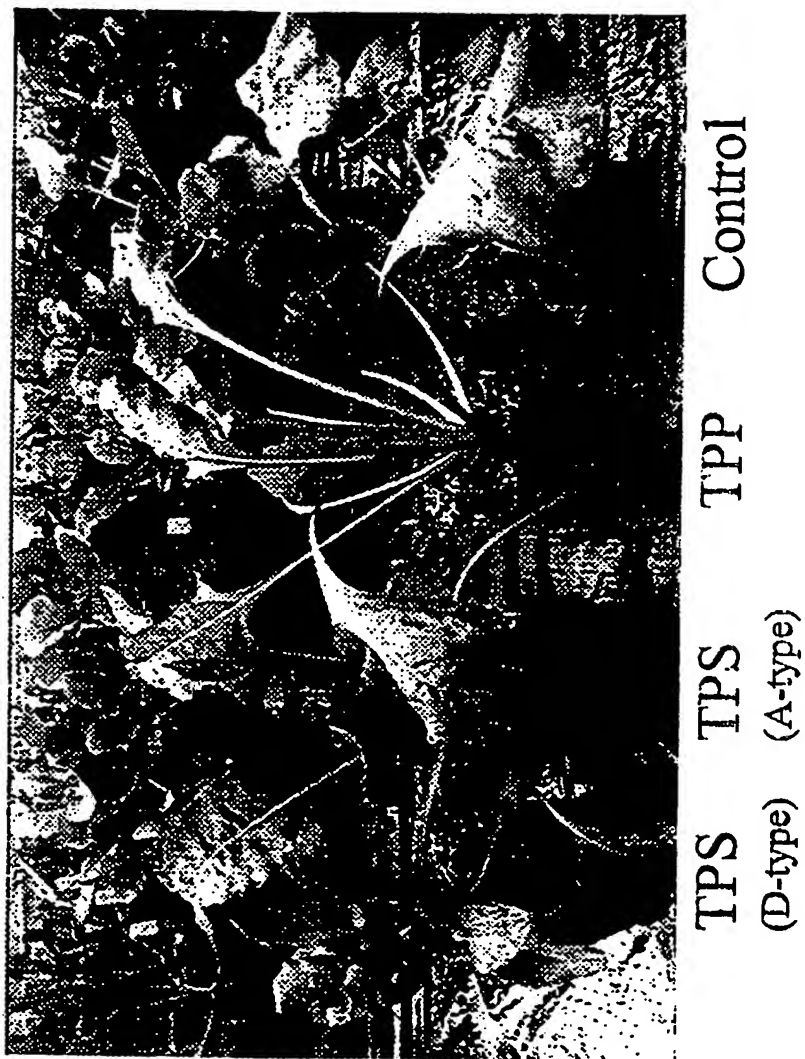


Fig. 21

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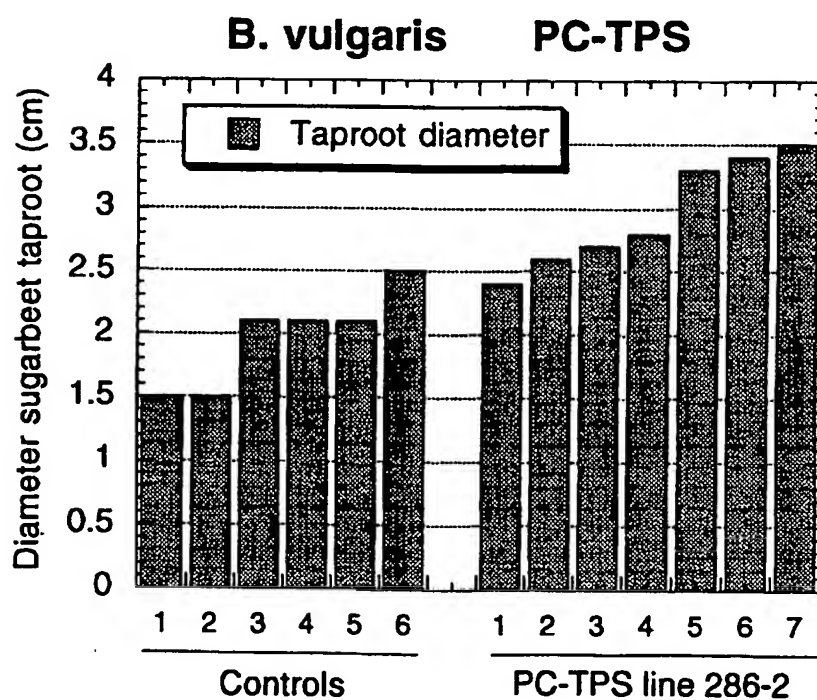
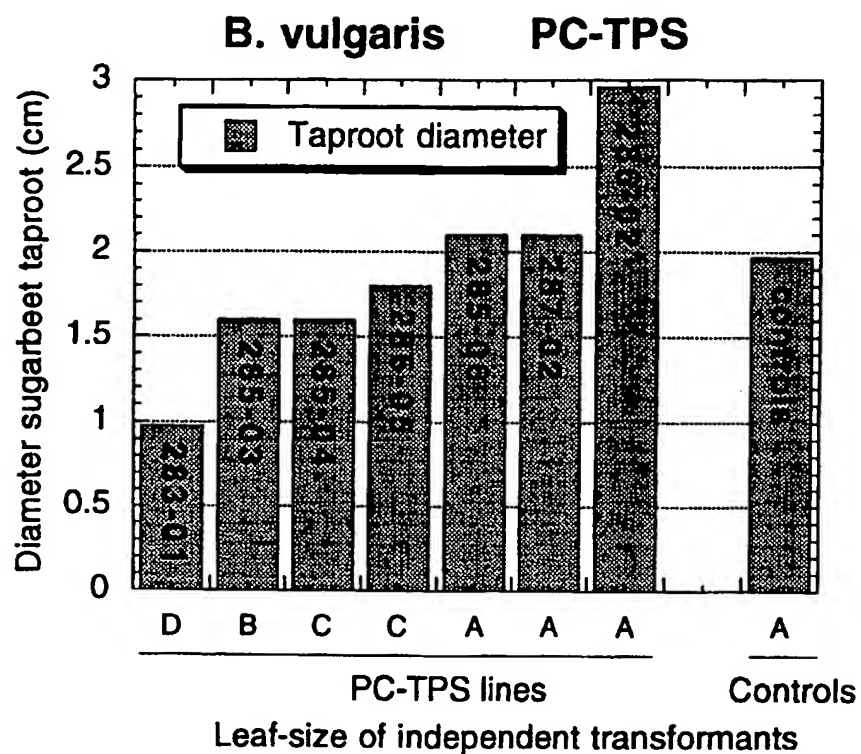


Fig. 22

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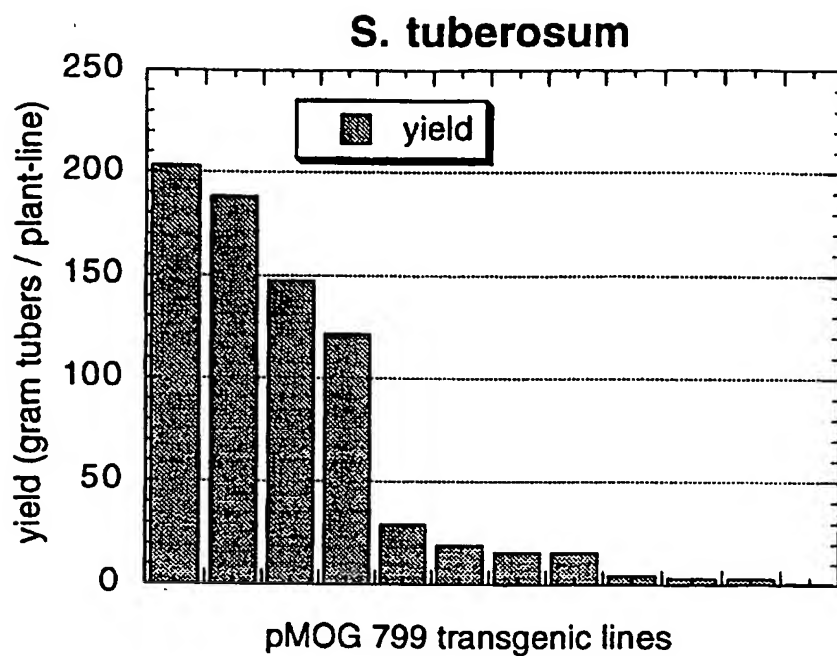


Fig. 23

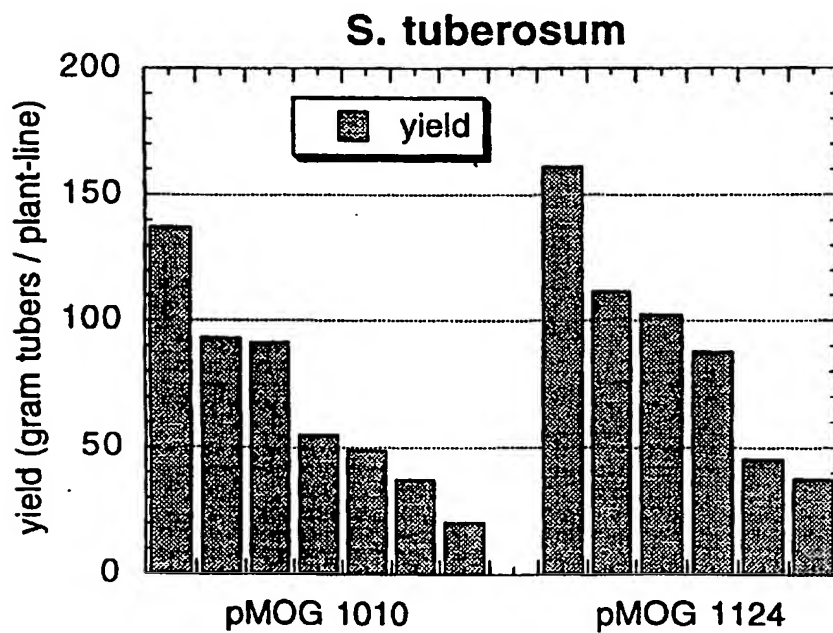


Fig. 24

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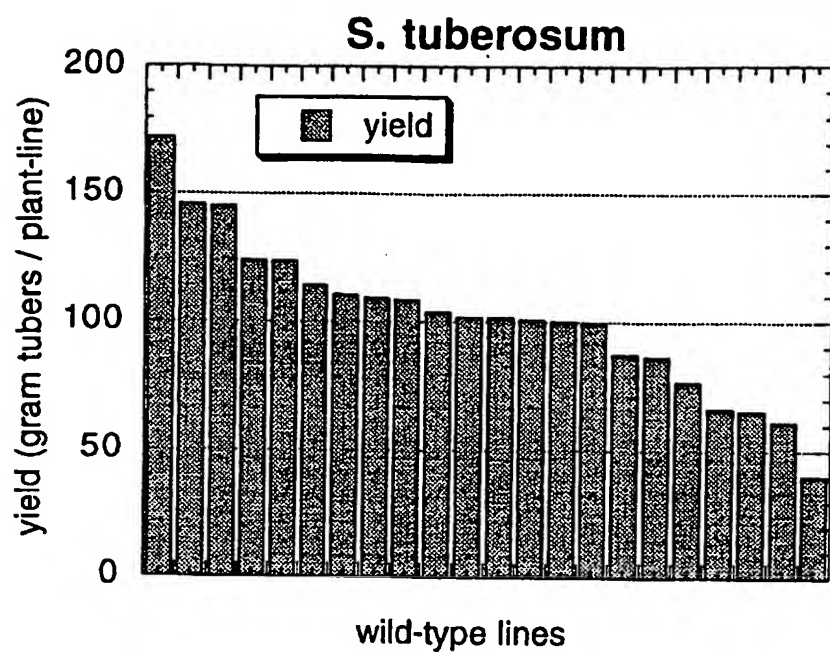


Fig. 25

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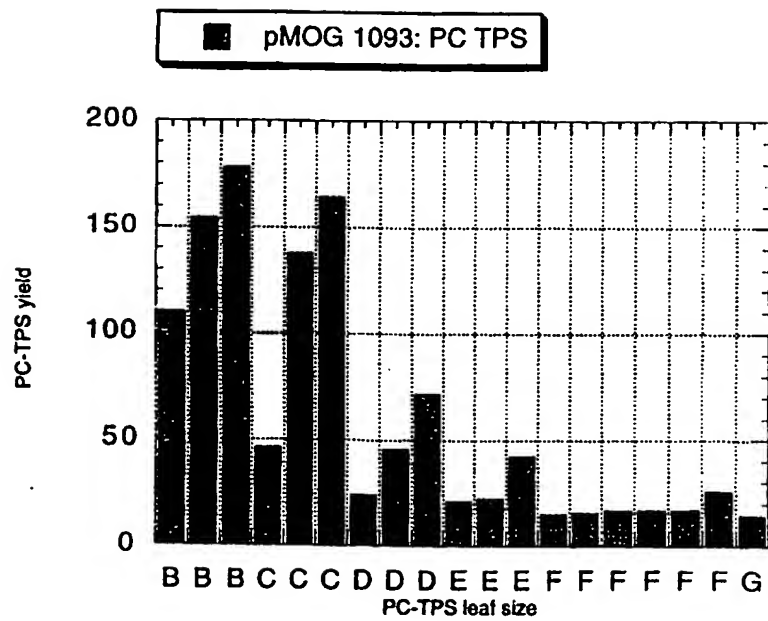
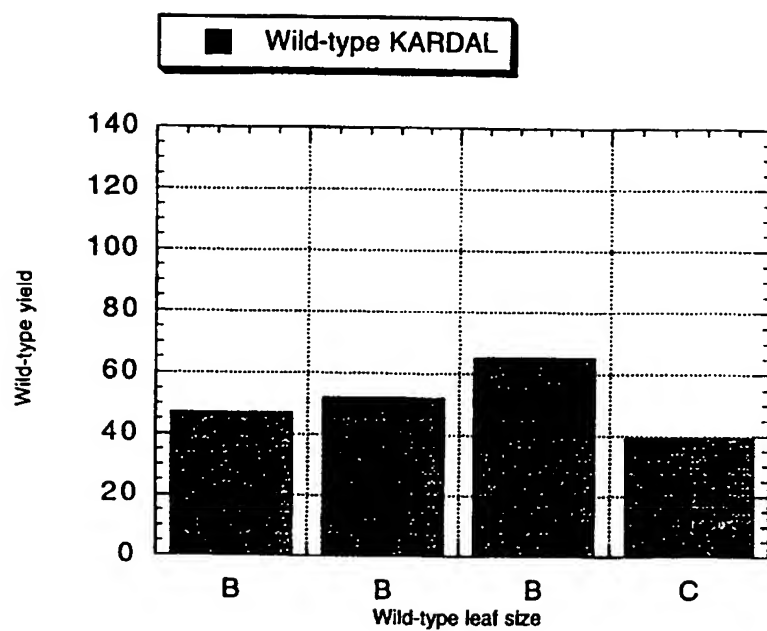
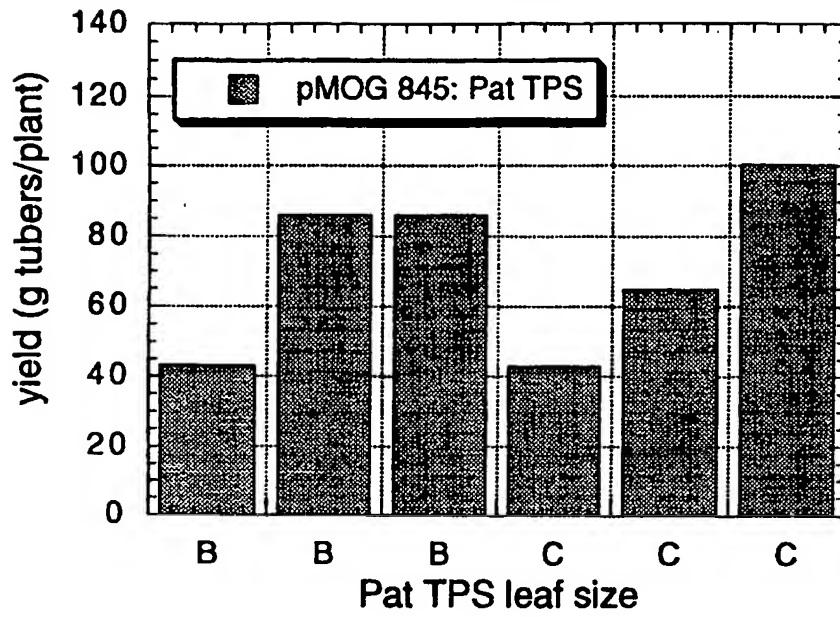
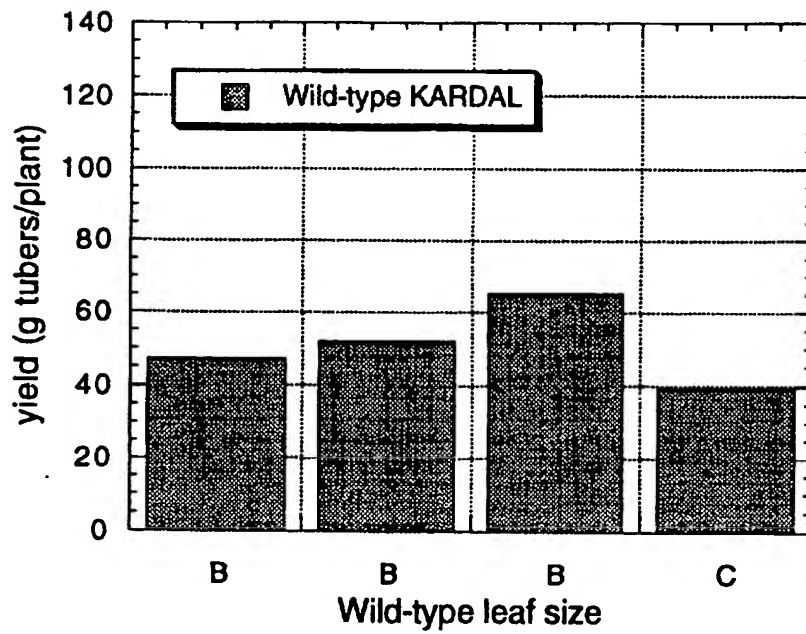


Fig. 26

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Potato**Potato****Fig. 27**

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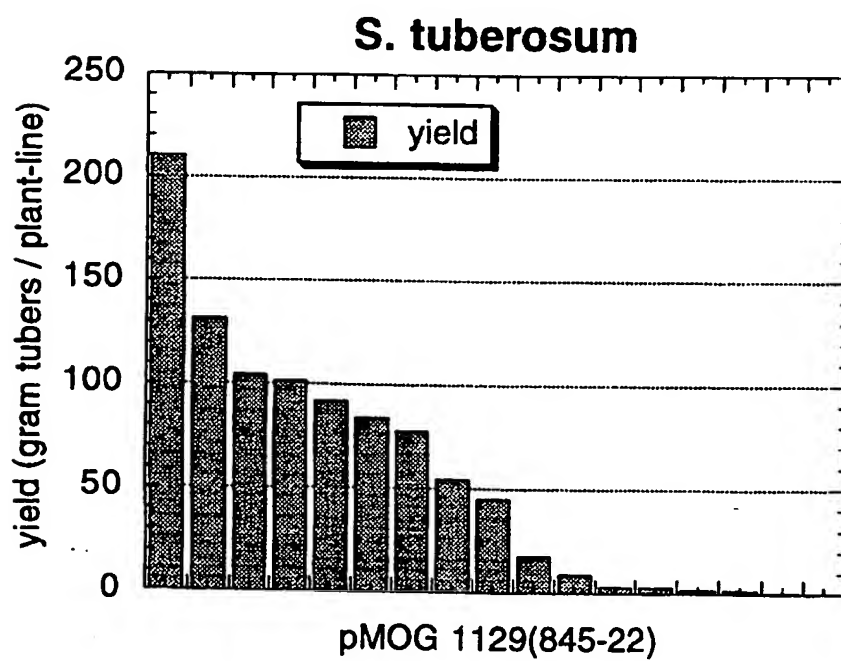
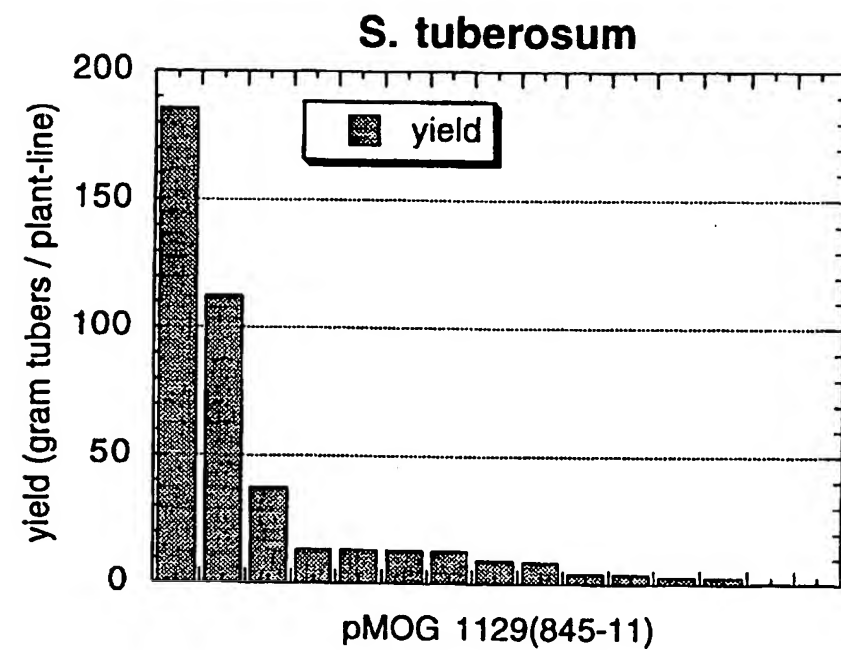


Fig. 28 (part 1)

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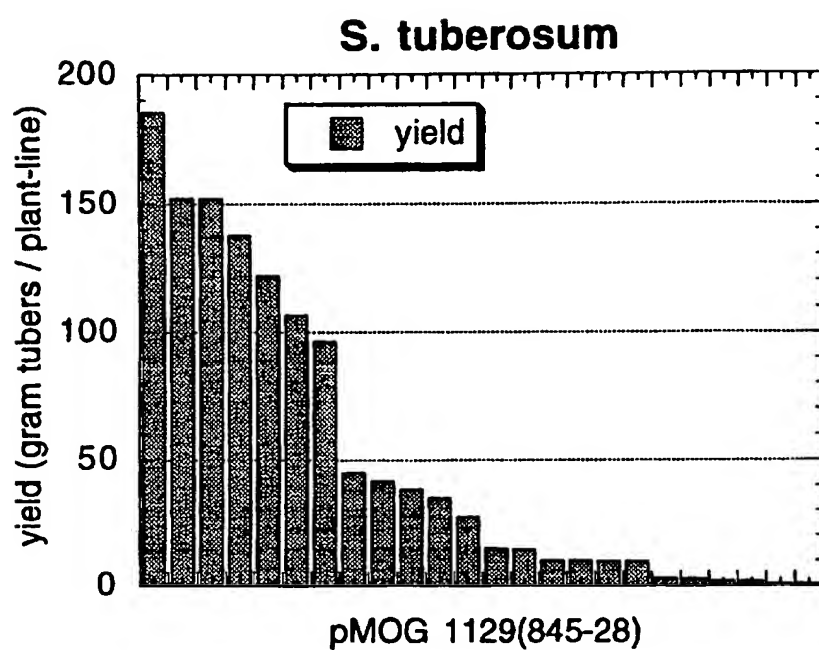
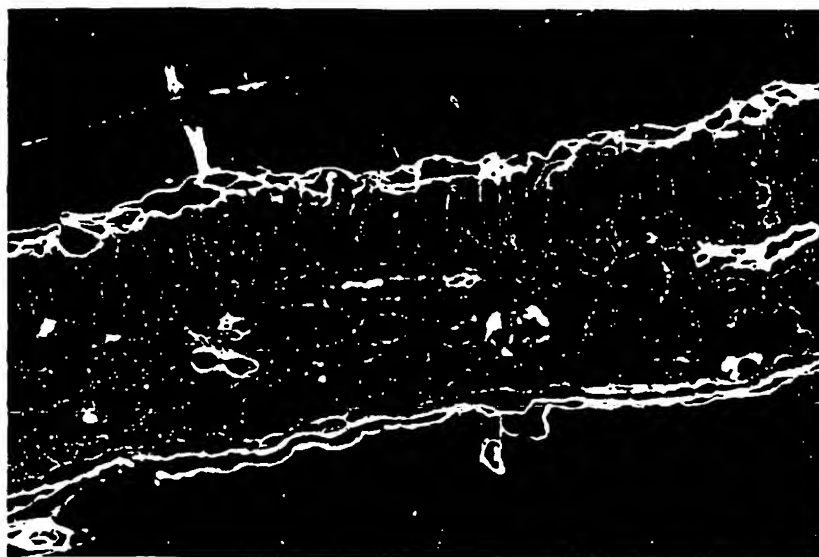


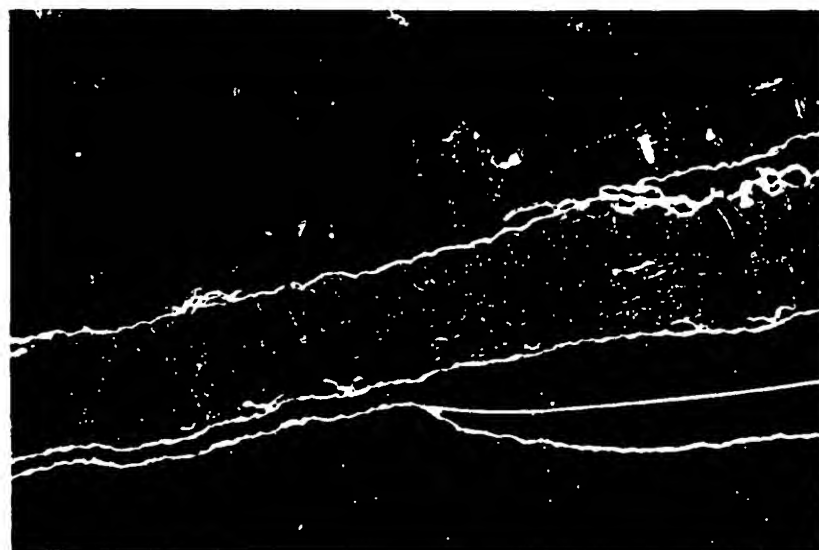
Fig. 28 (part 2)

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UPPER
EPIDERMIS
←
PALLISADE
MESOPHYLL
←
SPONGY
MESOPHYLL
←
LOWER
EPIDERMIS

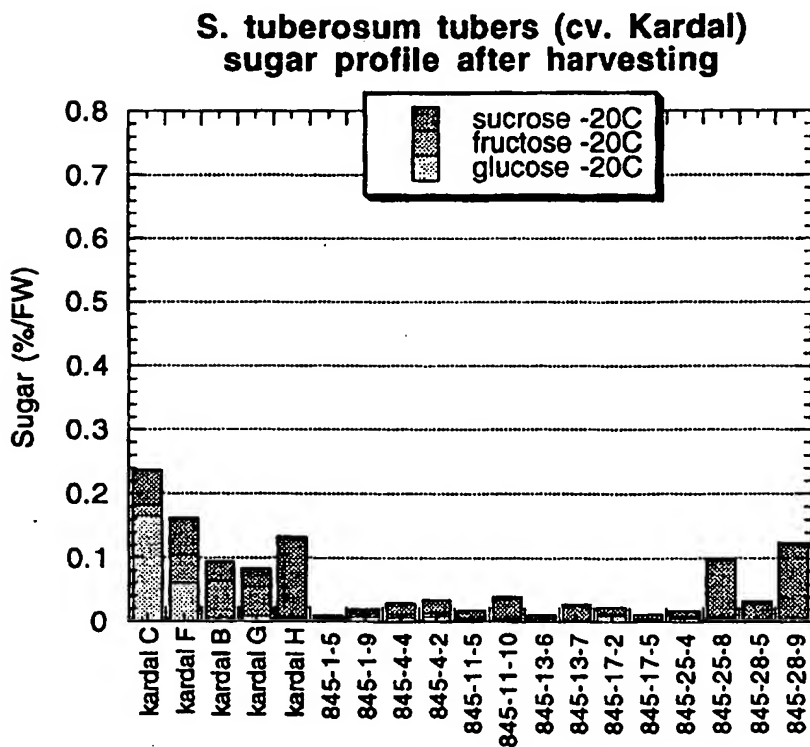
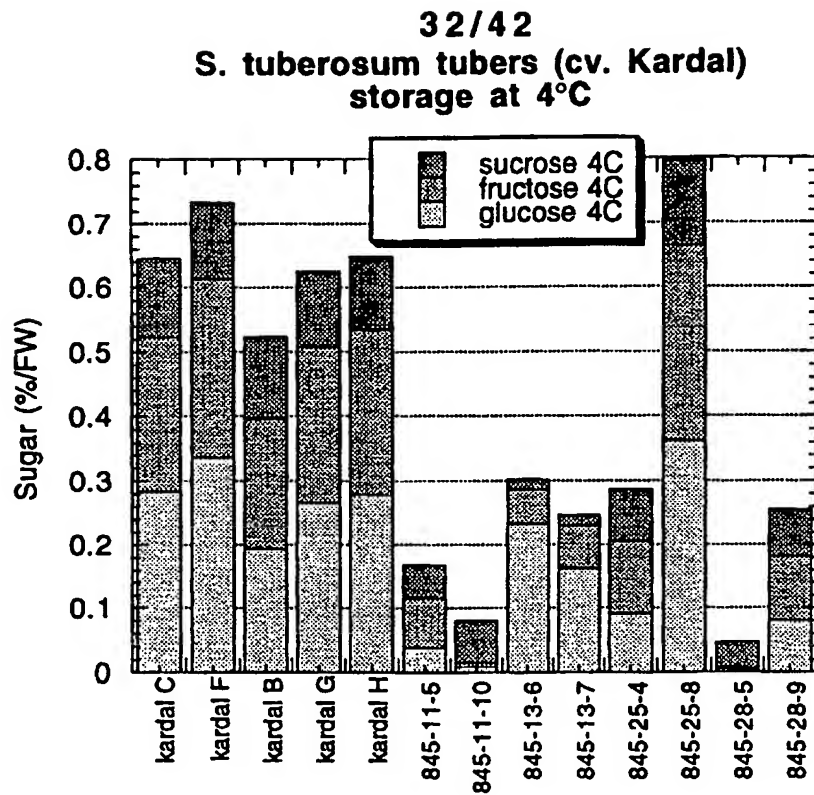
TPS TRANSGENIC TOBACCO LEAF



UPPER
EPIDERMIS
←
PALLISADE
MESOPHYLL
←
SPONGY
MESOPHYLL
←
LOWER
EPIDERMIS

TPP TRANSGENIC TOBACCO LEAF

Fig. 29

**Fig. 30**

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Fig. 31



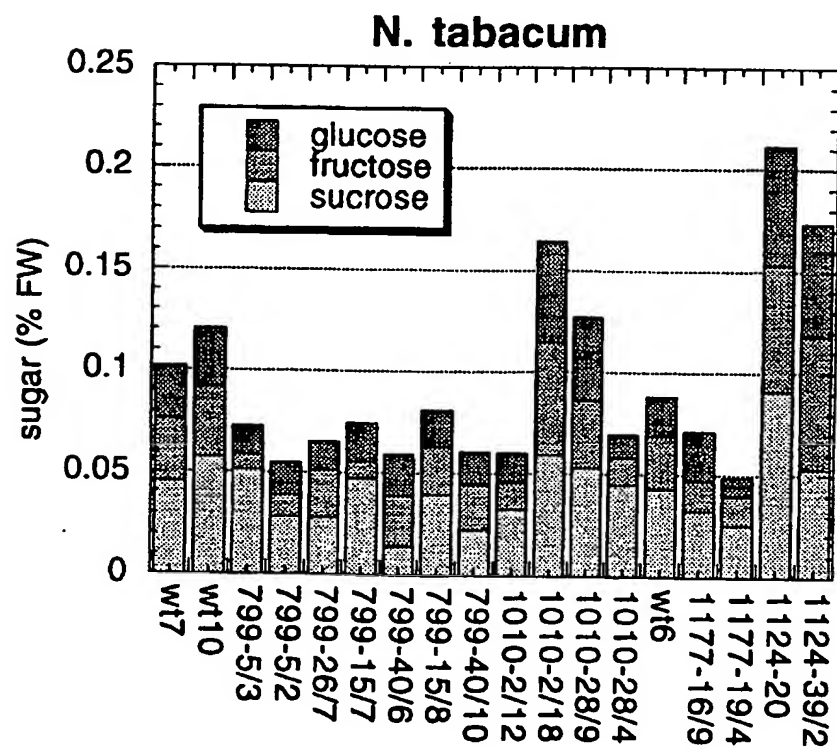
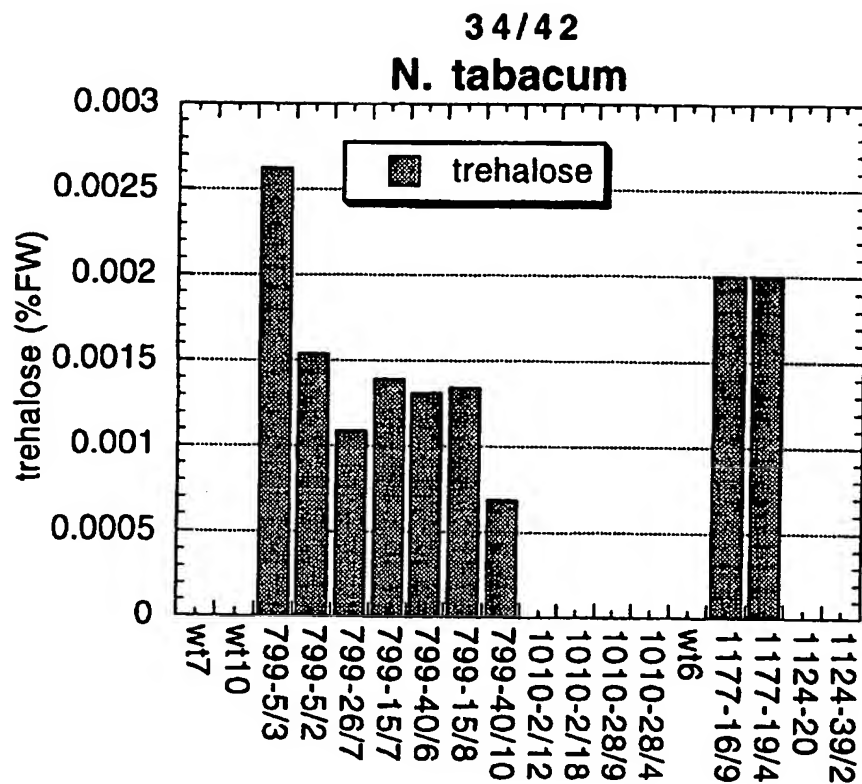


Fig. 32-1

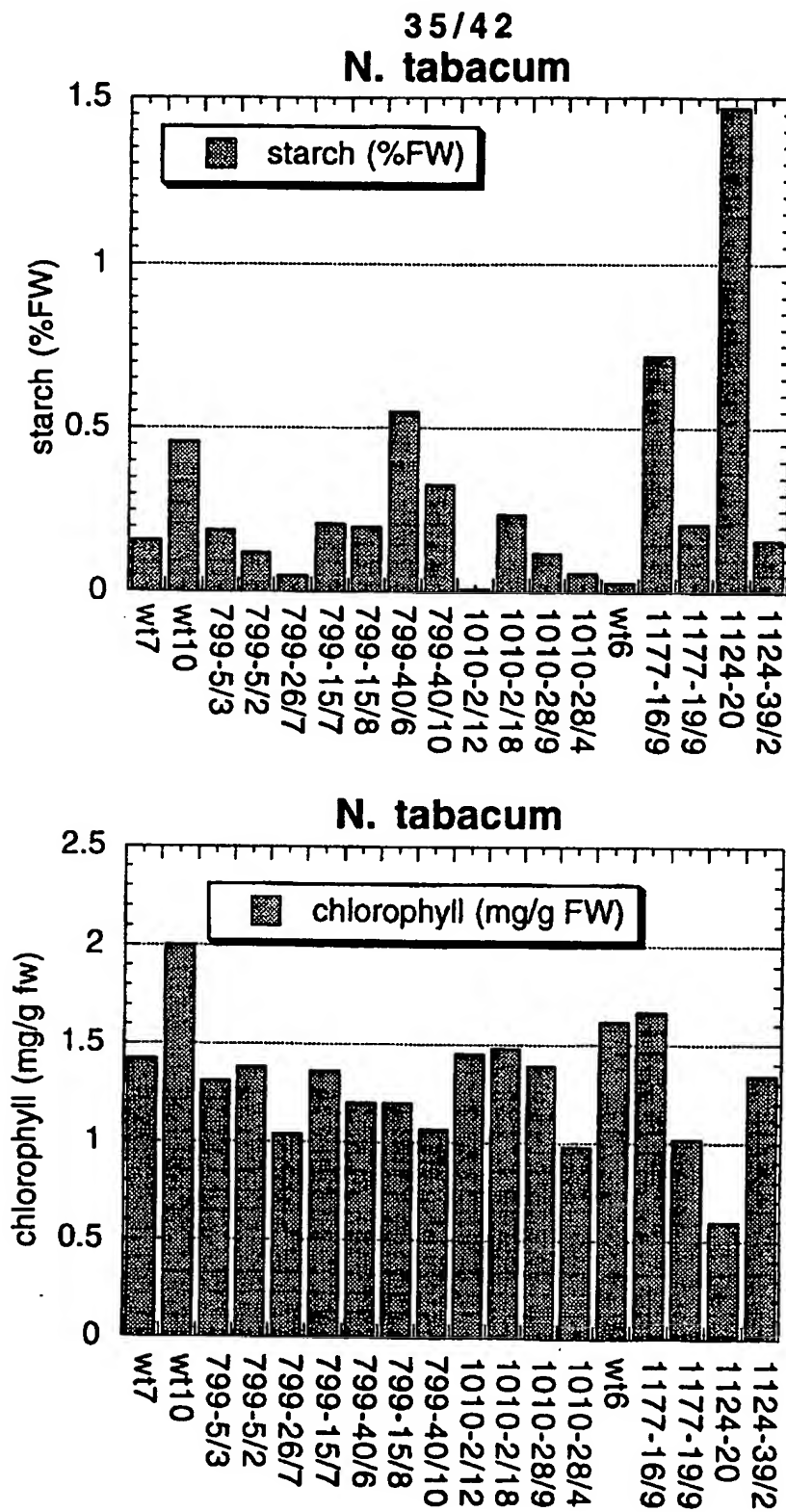


Fig. 32-2

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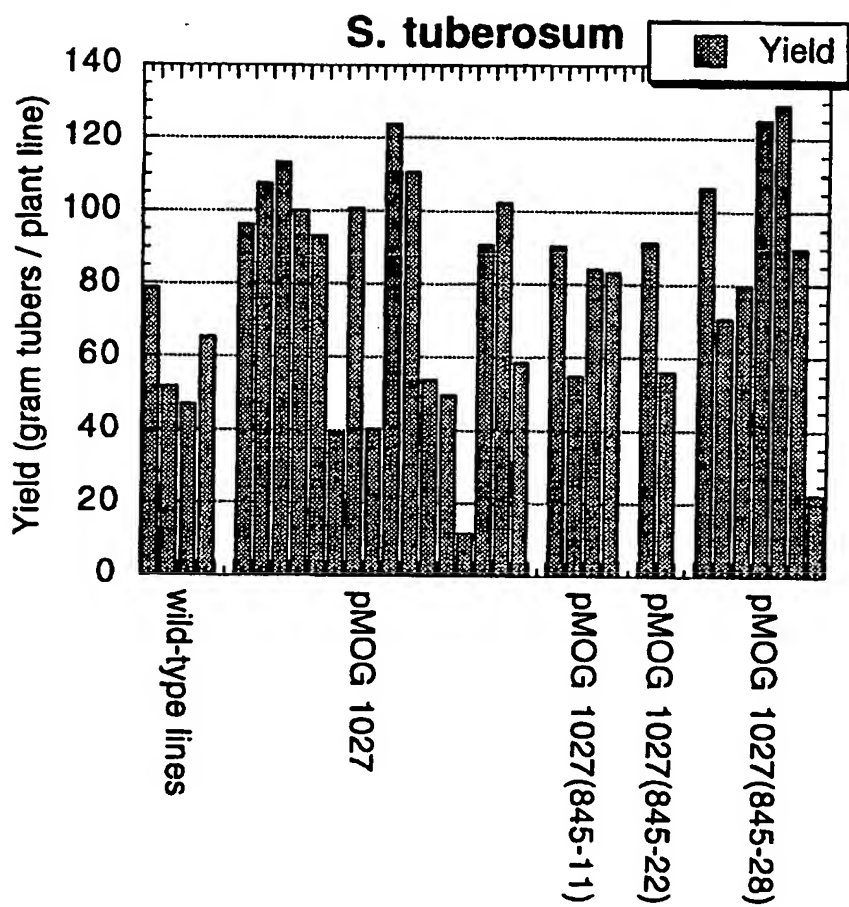


Fig. 33

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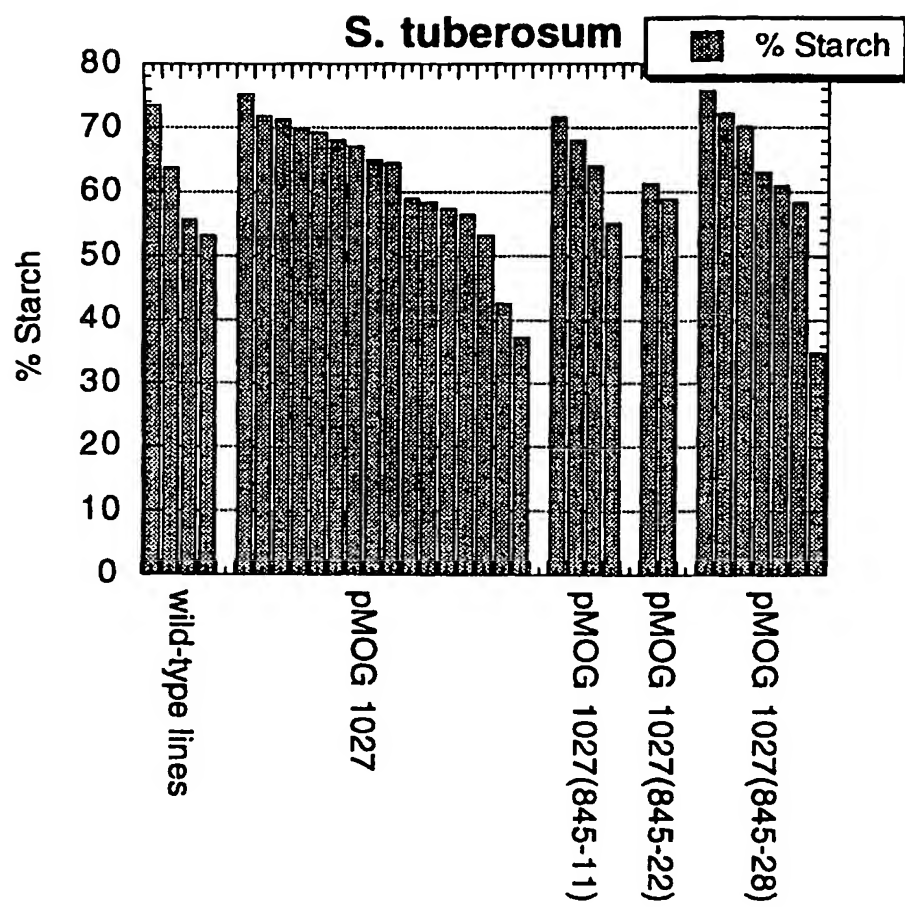


Fig. 34

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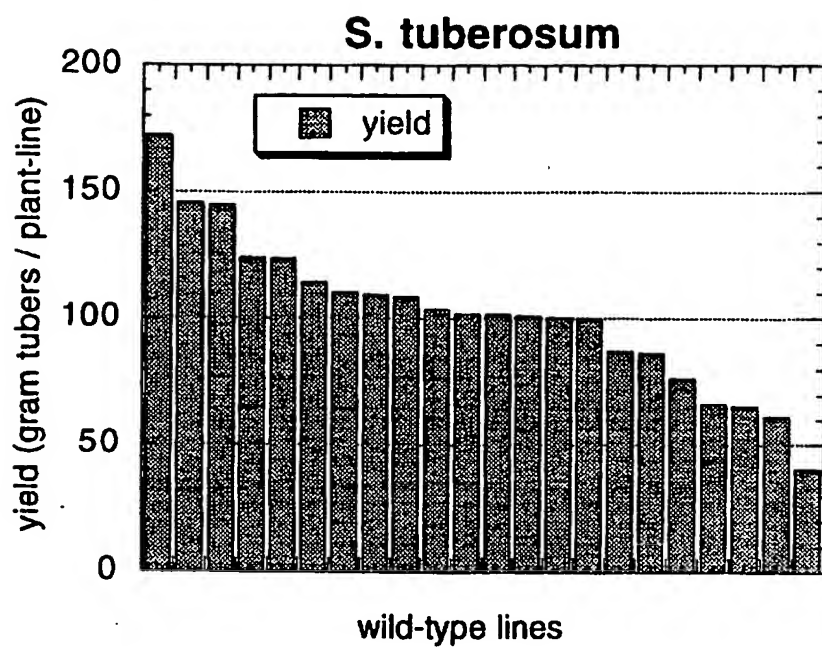
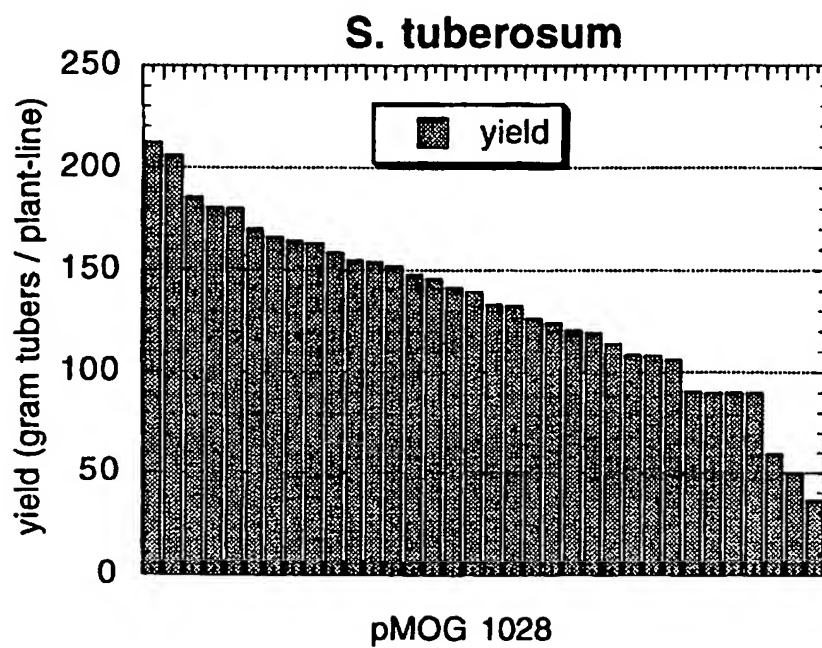


Fig. 35-1

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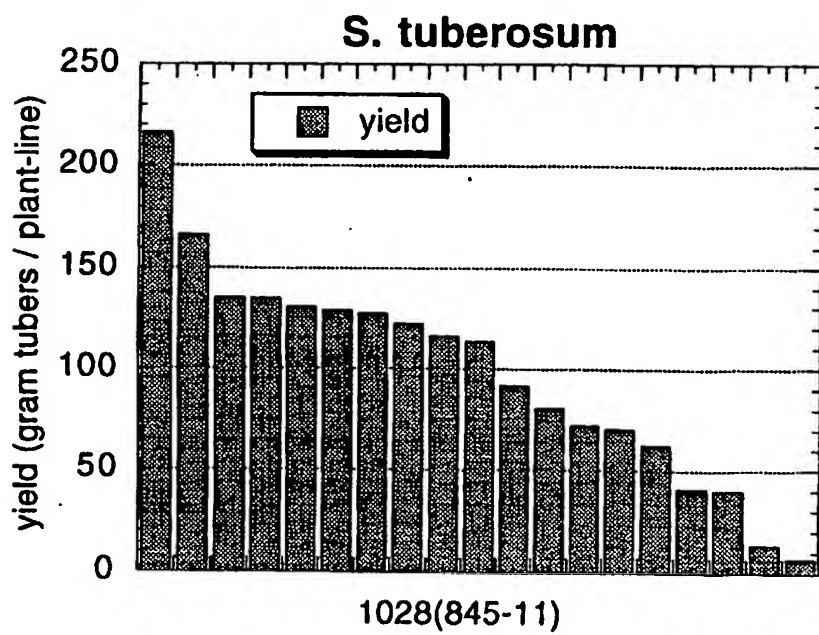
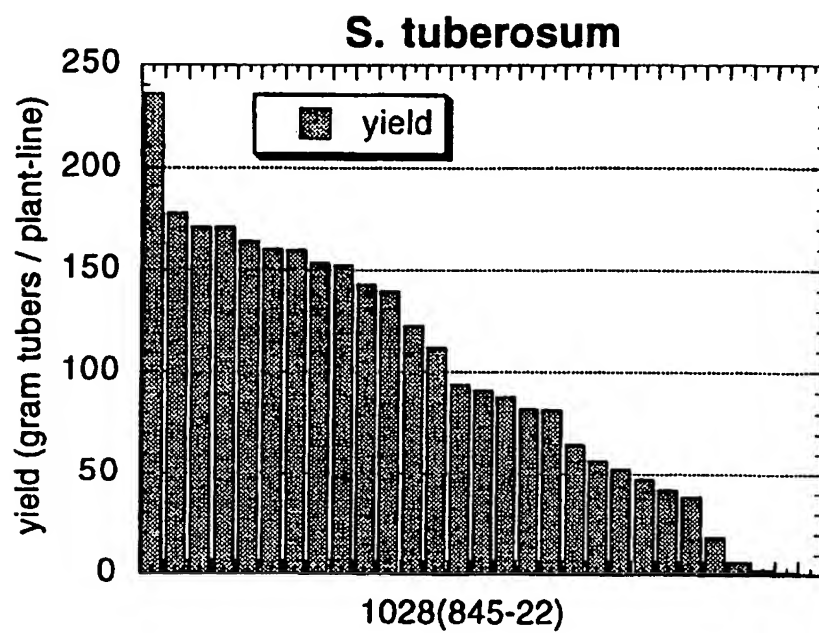


Fig. 35-2

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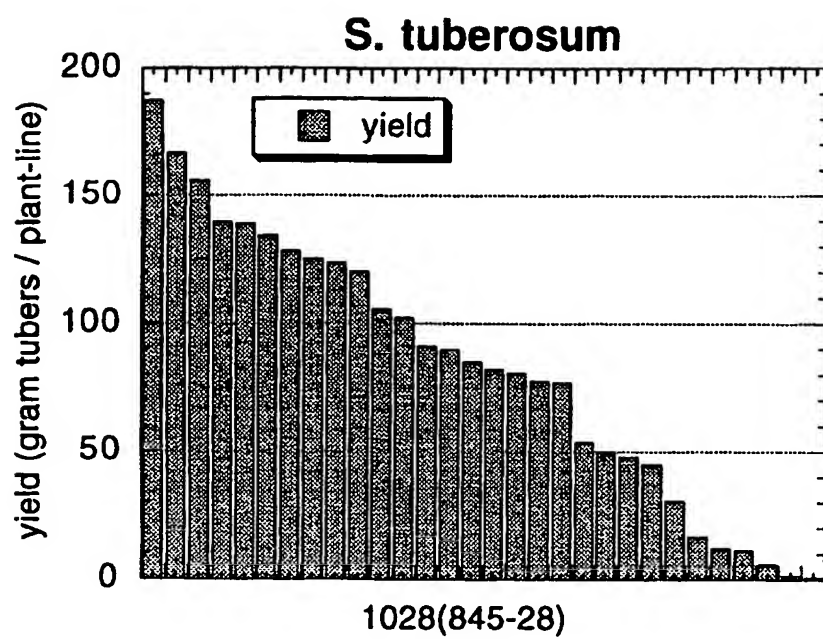


Fig. 35-3

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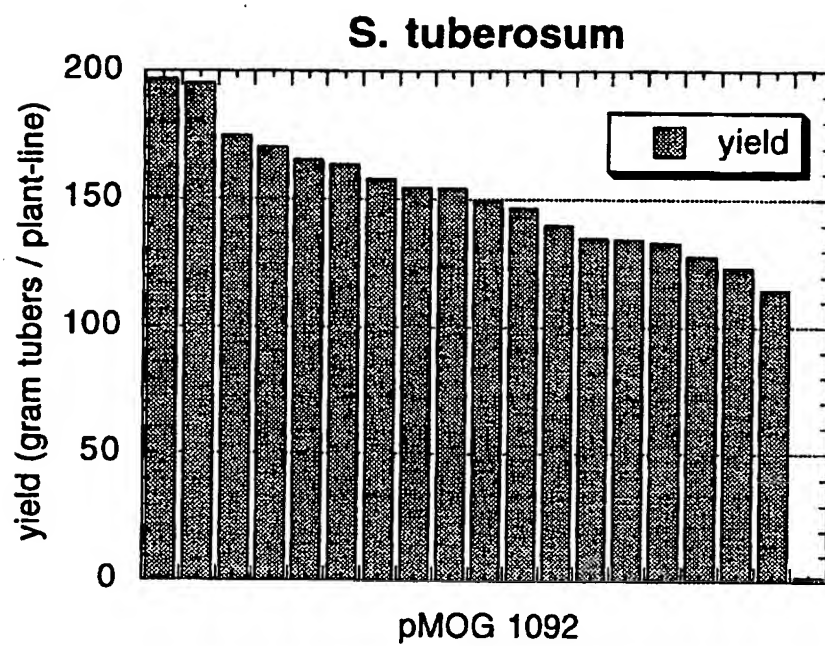


Fig. 36

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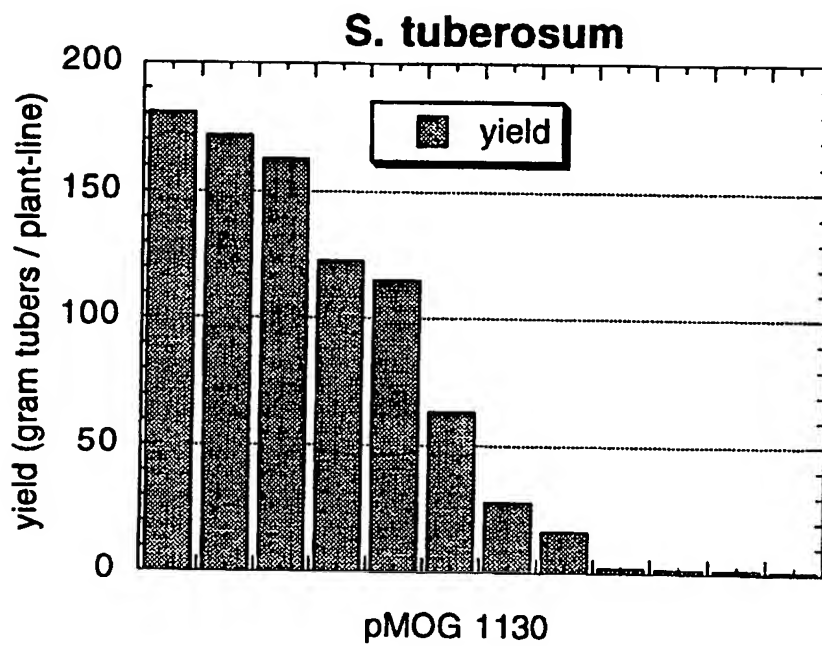


Fig. 37

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